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FRED MAAS ROLFS, 1872–1956

Walter W. Hansen, F. Ben Struble, and L. A. Brinkerhoff

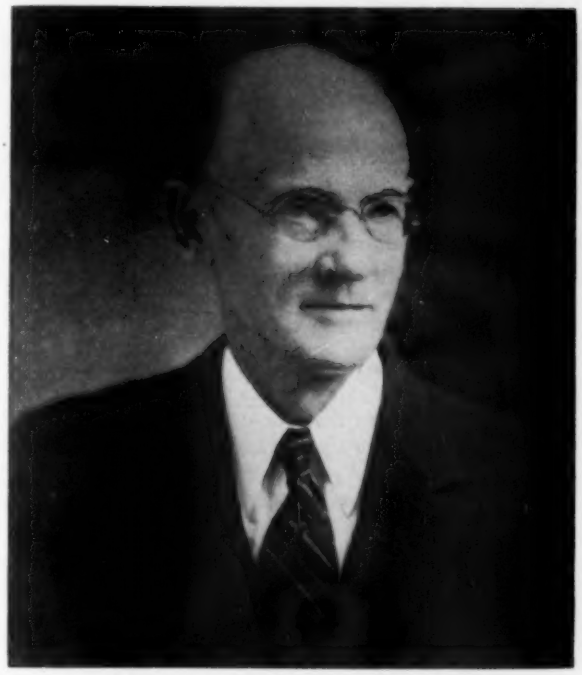
Fred Maas Rolfs, Professor Emeritus and former head of the Botany and Plant Pathology Department, Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma, died at his home in Stillwater after a short illness on February 8, 1956.

Dr. Rolfs was born at Le Claire, Iowa, on March 5, 1872, and was married to Adeline D. Evans at Boulder, Colorado, August 23, 1903. He spent his early years on his father's farm and attended the Iowa State College of Agriculture, from which he received his B.S. degree in 1897. He then moved to Fort Collins, Colorado, where he accepted a part-time position on the staff of Colorado State Agricultural College, thus continuing his research and education. He completed his M.S. degree in 1903. It was at this station that Dr. Rolfs published 2 important bulletins (Bulletins 90 and 91) dealing with Rhizoctonia on potatoes. He was among the pioneer workers on Rhizoctonia on potato in America, and was the first to demonstrate the relation of the basidiomycetous stage (*Corticium vagum* var. *solani*) to the imperfect stage (*Rhizoctonia*).

From 1903 to 1906 Dr. Rolfs was Professor of Botany and Horticulture at the University of Florida. From 1906 to 1910 he was Plant Pathologist at the Missouri State Fruit Experiment Station. Here he published 2 bulletins, "Fruit Tree Diseases and Fungicides" and "Winter Killing of Twigs, Cankers, and Sun Scab of Peach Trees." He entered Cornell University in 1911 and began work toward his Ph.D. degree, which he received in 1913. His thesis, "A Bacterial Disease of Stone Fruits," subsequently was published as a Memoir. This was the first monographic study of bacterial spot of stone fruits and was one of the early comprehensive investigations of a bacterial disease of plants. Although certain of Rolfs' conclusions on bacterial spot have necessarily been altered in the light of later investigations, his work still serves as a basis for further studies of this disease.

He was Associate Professor of Botany, Plant Pathology, and Bacteriology at Clemson Agricultural College of South Carolina from 1913 to 1915. While at Clemson, he completed the work for one of his outstanding publications, "Angular Leaf Spot of Cotton" (Bulletin 184). In this investigation he laid the foundation of our understanding of the life history of the pathogen, emphasized the importance of seed as a source of inoculum, and demonstrated that the causal bacterium survives the winter in or on the seed and on the lint. He also showed that the disease could be greatly reduced by treating the seed either with sulfuric acid or with hot water.

In 1915 Dr. Rolfs moved to Stillwater, Oklahoma, where he was Professor of Horticulture at the Oklahoma Agricultural and Mechanical College from 1916 to 1923. In 1923 he became head of the Botany and Plant Pathology Department and remained in this position until he retired in 1937. While at Stillwater, he continued to work with diseases of cotton and published circulars and short articles. His last publication, "Apple Blotch," was in 1942 (Bulletin B-261); however, Dr. Rolfs remained active in research and was especially interested in angular leaf spot of cotton until the time of his death.



FRED MAAS ROLFS, 1872–1956

Survivors include his wife and 2 foster daughters, Miss Francis M. Fentress of Tulsa, Oklahoma, and Mrs. G. W. Phillips of Annapolis, Maryland. Dr. Rolfs was the brother of Dr. Peter H. Rolfs, the first director of the Florida Agricultural Experiment Station (1906) and also one of the pioneer plant pathologists of America. Both were charter members of The American Phytopathological Society.

Dr. F. M. Rolfs was a member of the St. Andrews Episcopal Church. He was a member of the following scientific organizations: The American Phytopathological Society, A.A.A.S. (Fellow), Botanical Society of America, Ecological Society of America, Oklahoma Academy of Science, Phi Kappa Phi, Pi Gamma Mu, Phi Sigma, and Sigma Xi. He is listed in Who's Who in America.

PHYTOPATHOLOGY for July (46: 299-412) was issued July 21, 1956

THE GENETIC BASIS OF THE CROWN RUST RESISTANCE OF THE OAT VARIETY ASCENCAO [1]

M. D. Simons [2]

SUMMARY

Segregating populations resulting from crossing the oat variety Ascencao with various resistant and susceptible varieties were tested for reaction to *Puccinia coronata* Cda. var. *avenae* Fraser & Led. and to *Helminthosporium victoriae* Meehan & Murphy. The data obtained indicated that Ascencao carried the Victoria gene, or gene complex, for reaction to the crown rust pathogen and to *H. victoriae*, which accounted for its susceptibility to *H. victoriae* and its moderate resistance to race 263 of the crown rust fungus. In addition to the Victoria gene, Ascencao also posessed a single previously undescribed gene, designated "E", which was epistatic to the Victoria gene as far as reaction to the crown rust fungus was concerned, and which, either alone or in combination with the Victoria gene, conditioned an immune seedling reaction to available isolates of the common North American races of the crown rust fungus. This gene conferred a higher type of resistance than that shown by Landhafer, Santa Fe, or Trispernia, and apparently was not allelic to genes carried by these varieties.

In an earlier paper (6), the use of pathological techniques to distinguish genetically different sources of resistance to crown rust of oats (caused by *Puccinia coronata* Cda. var. *avenae* Fraser & Led.) was discussed. The variety Ascencao was characterized by its immune reaction to available isolates of common races of the crown rust pathogen, and its susceptibility to *Helminthosporium victoriae* Meehan & Murphy. Although indicating some kinds of genetic differences from previously known sources of resistance to crown rust, these pathologic data gave no indication of the genetic complexity of the reactions involved, nor whether the genes conditioning the reaction of Ascencao were allelic to genes carried by previously studied varieties. The purpose of this paper is to report the results of investigations undertaken in an attempt to answer these questions.

Literature on the inheritance of reaction to the crown rust fungus recently has been reviewed by Finkner, Atkins, and Murphy (2). The general consensus of the various papers mentioned was that the resistance of Landhafer was conditioned by a single gene, whereas the resistance of Trispernia and Santa Fe was conditioned by 1 or more genes, with individual plants apparently differing in genetic constitution. Finkner (3) recently reviewed the literature on inheritance of reaction to *H. victoriae* and its relationship to reaction to the crown rust fungus. Susceptibility to *H. victoriae* and resistance to the crown rust fungus are both monogenic characters, and are very strongly linked in the coupling phase. Reactions to these 2 diseases also have been explained as a pleiotropic effect of a single gene.

[1] Accepted for publication March 1, 1956.

Cooperative investigation between the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Iowa Agricultural Experiment Station. Journal paper No. J-2865 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1176.

[2] Plant Pathologist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

MATERIALS AND METHODS.—The varieties of oats (*Avena sativa* L.) used in this study included Markton and Clinton, which are susceptible to the common races of the crown rust fungus, and Ascencao, Victoria, Landhafer, Trispernia, and Santa Fe, which are resistant to the common races. Races 202 (stock culture No. 2) and 263 (stock culture No. 18) of *P. coronata* var. *avenae* were used for testing reactions of segregating progenies. Techniques of inoculation, isolation, and increase of races and notation of reaction type were similar to those described by Murphy (4) and Finkner, Atkins, and Murphy (1).

The culture of *H. victoriae* used was isolated from Tama oats grown at Ames, Iowa. Inoculation was accomplished by rubbing or spraying mycelial mats, ground in water, onto the leaves. The plants were then kept in a humidity chamber about 24 hours. Observations were made 2 days later.

EXPERIMENTAL RESULTS.—*Inheritance of reaction to H. victoriae.*—Ten F_2 populations involving, as parents, Ascencao and several varieties resistant to *H. victoriae*, were tested for reaction to *H. victoriae*. In all cases these populations segregated in the manner expected under the assumption of monogenic inheritance, with susceptibility to the pathogen being dominant over resistance.

Inheritance of reaction to the crown rust fungus.—1) *Ascencao* × *Markton.*—A hypothesis was formulated in which it was assumed that Ascencao carried the dominant, Victoria gene, or gene combination, for susceptibility to *H. victoriae* and moderate resistance to the crown rust fungus, plus another dominant gene for immunity to the crown rust fungus that was epistatic to the Victoria gene insofar as reaction to the crown rust fungus was concerned. The phenotypes expected in an F_2 population from a cross of Ascencao and any variety susceptible to crown rust are shown in Table 1. Among those susceptible to *H. victoriae*, the separation of classes immune from crown rust and those characterized by flecking reactions is difficult. Consequently these 2 classes are combined into a single phenotypic class.

TABLE 1.—*Observed and expected frequencies of phenotypic classes for a 1:3:2:1:9 ratio for reaction to race 202 of the crown rust fungus and to H. victoriae in an F_2 population from a cross of Ascencao[a] and Markton[b]*

Reaction			
Crown rust	*H. victoriae*	Observed	Expected
Susceptible	Resistant	10	11.9
Moderately resistant	Susceptible	44	35.8
Highly resistant	Resistant	18	23.9
Immune	Resistant	14	11.9
Highly resistant and immune	Susceptible	105	107.5
Total		191	
Chi-square		4.041	
Probability		0.5–0.3	

[a] Immune to race 202 and susceptible to *H. victoriae*.
[b] Susceptible to race 202 and resistant to *H. victoriae*.

One F_2 population from a cross of Ascencao and the variety Markton, which is susceptible to crown rust, was tested in the first-leaf stage for reaction to race 202 of the crown rust fungus. The same plants were then tested in the second-leaf stage for reaction to *H. victoriae*, to which Markton is resistant. The number of individuals observed in the various phenotypic classes did not vary significantly from the numbers expected under the hypothesis outlined above (Table 1). The class characterized as resistant to *H. victoriae* and immune from crown rust was reasonably clear-cut. At other times, 2 other F_2 populations from crosses of Ascencao and Markton and 1 population from a cross of Ascencao and the susceptible variety Clinton were tested in the same way. The results were essentially similar, except that the classes resistant to *H. victoriae* and characterized by either immune or by flecking reactions to the crown rust fungus were not so clear-cut.

2) *Ascencao × Victoria.*—Three F_2 populations from crosses of Ascencao and Victoria were tested for reaction to race 202 of the crown rust pathogen, but not for reaction to *H. victoriae*. Under the hypothesis, no susceptible plants would be expected, since they all should carry the Victoria gene. Only the Ascencao gene should segregate, and this was found to be the case. About ¼ of the F_2 plants showed the typical, necrotic, moderately resistant reaction of Victoria. The remainder were uniformly immune, indicating that in these crosses, and under the particular conditions under which the plants were tested, the other gene carried by Ascencao was epistatic to the Victoria gene in both the heterozygous and homozygous states.

3) *Reaction to race 263 of the crown rust fungus.*—The rare race 263 parasitizes such varieties as Landhafer and Santa Fe, which are used as sources of resistance to common races (5). Victoria is resistant to this race, showing the typical necrotic reaction. Ascencao also is resistant, but does not show the immune reaction. Instead, it reacts like Victoria with production of small uredia surrounded by a necrotic ring.

Plants of an F_2 population from a cross of Ascencao and Santa Fe were tested for reaction to race 263 and to *H. victoriae*. About ¼ of the F_2 plants were clearly resistant to *H. victoriae*, whereas the remainder were definitely susceptible. Those plants resistant to *H. victoriae* were, without exception, susceptible to race 263. Those plants susceptible to *H. victoriae* were all resistant to race 263, most of them showing the typical Victoria reaction. These data further substantiated the presence of the Victoria gene in Ascencao and indicated that the other gene in Ascencao conferred no resistance to race 263.

4) *Ascencao crossed with crown-rust-resistant varieties.*—Since the Ascencao gene conditioning an immune response to common races differs in phenotypic expression from previously known genes for reaction to the crown rust pathogen, it obviously is not identical to any of them. This leaves 2 possibilities. First, the Ascencao gene may simply be allelic to 1 of the previously known genes. In this case it could not be combined in the same variety with the previously known gene. Second, it may represent a new locus. In order to obtain information on this point, Ascencao was crossed with the previously known resistant varieties Victoria, Landhafer, Santa Fe, and Trispernia. Crosses involving Victoria have been discussed above. Segregating populations from crosses of Ascencao and the other 3 varieties were tested for reaction to the common race 202 of the crown rust fungus. Because of the relatively large number of resistant genes involved and the consequent intergradation of reaction types, it was extremely difficult to analyze the data on a factorial basis. All the F_2 populations tested, however, contained at least a few plants that were completely susceptible, indicating that the Ascencao gene was not allelic to any of the genes carried by the plants of Landhafer, Santa Fe, or Trispernia that were used in making the crosses.

5) *Reactions of F_3 and F_4 populations.*—Plants of the F_2 population of the Ascencao and Markton cross shown in Table 1 were transplanted to individual pots so that they could be grown to maturity and thus provide a representative F_3 population with which the results obtained in the F_2 generation could be corroborated. Unfortunately, most of the plants that had been susceptible to *H. victoriae* either died prematurely or produced little or no viable seed. Progenies from plants that had been resistant to *H. victoriae* were tested for reaction to *H. victoriae* and to race 202 of the crown rust fungus. All of these lines were resistant to *H. victoriae*, and some were apparently breeding true for the immune type of reaction to race 202. A few of these true-breeding lines were grown to maturity, and the resulting F_4 progenies also proved to be immune.

Designation of the Ascencao gene.—It is proposed that the gene carried by the variety Ascencao, a gene that is characterized by its epistasis to the Victoria gene and by its conditioning an immune type reaction

to the common races of the crown rust pathogen, be designated by the letter "E." The use of this symbol will prevent confusing the Ascencao gene with previously designated genes for reaction to the crown rust fungus or to *Puccinia graminis* Pers. f. sp. *avenae* Erikss. & E. Henn., the cause of stem rust of oats.

Discussion.—The unusually high type of resistance conferred by the "E" gene under greenhouse conditions suggests that this gene might be useful in breeding new varieties of oats resistant to crown rust. Little is known, however, about the degree of protection afforded by this gene under field conditions, and further testing will be required to determine its value as a source of crown rust resistance in breeding programs.

Apparently the "E" gene was not allelic to any of the genes carried by plants of other resistant varieties that were used in making the crosses studied. In view of the intravarietal genetic variability of these varieties, however, it is difficult to prove positively that a given gene is not allelic to any of the genes that might be found in these variable varieties.

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FERTILIZER INJURY AND ITS RELATIONSHIP TO SEVERAL PREVIOUSLY DESCRIBED DISEASES OF LETTUCE[1]

R. G. Grogan and F. W. Zink

SUMMARY

A disease of lettuce that has caused considerable damage to commercial plantings in California was compared with several previously described diseases of lettuce. The present disease was reproduced by the application of toxic concentrations of inorganic commercial fertilizer materials or of animal manure. A comparison of the relative toxicity of the several fertilizers revealed that those containing ammonium hydroxide and free ammonia were most toxic but that certain other materials containing nitrites, nitric acid, or phosphoric acid also were toxic. Materials composed of neutral salts such as ammonium sulfate, ammonium nitrate, or calcium nitrate were relatively innocuous. An explanation of the etiology of the previously described diseases on the basis of toxic nitrogen compounds formed during the decomposition of organic nitrogenous materials in the soil was suggested.

A disease of lettuce of hitherto undetermined etiology has caused considerable damage in the Salinas Valley of California during recent years. Several reports of lettuce diseases with somewhat similar syndromes have been made from other areas of the United States (1, 3, 4, 5, 8, 11, 13, 20, 22). In 2 of these reports the disorder was attributed to the action of bacteria, and 2 new species were described (1, 22). In the other reports the cause was either not designated or was attributed to the action of *Pythium* spp. A similar disease was described by Selby (20) and attributed to the action of Rhizoctonia, but no proof of this relationship was presented.

In several of these reports the disease incidence was reported to be increased by cold and/or wet soil conditions (4, 5, 8). Brown (1) reported in some detail the correlation of disease incidence and the previous application of fresh manures. Marlatt (11) also reported a correlation between the incidence of "brown stele" and the heavy application of manure.

In California, the occurrence of the disease appeared to be correlated somewhat with cold waterlogged soil conditions and/or with moderate to heavy applications of commercial fertilizers or manures.

The present study was initiated to obtain more specific information concerning the cause of the disorder and its relationship to fertilization practices.

Description of symptoms.—Characteristic above ground symptoms include wilting, which may be temporary and confined to the outer frame leaves (Fig.

[1] Accepted for publication March 5, 1956.

1, A) or, in more severe cases, the plant may die. The leaves of affected plants are usually a lusterless dark or gray-green color. The outer frame leaves sometimes develop yellowed V-shaped sectors that later

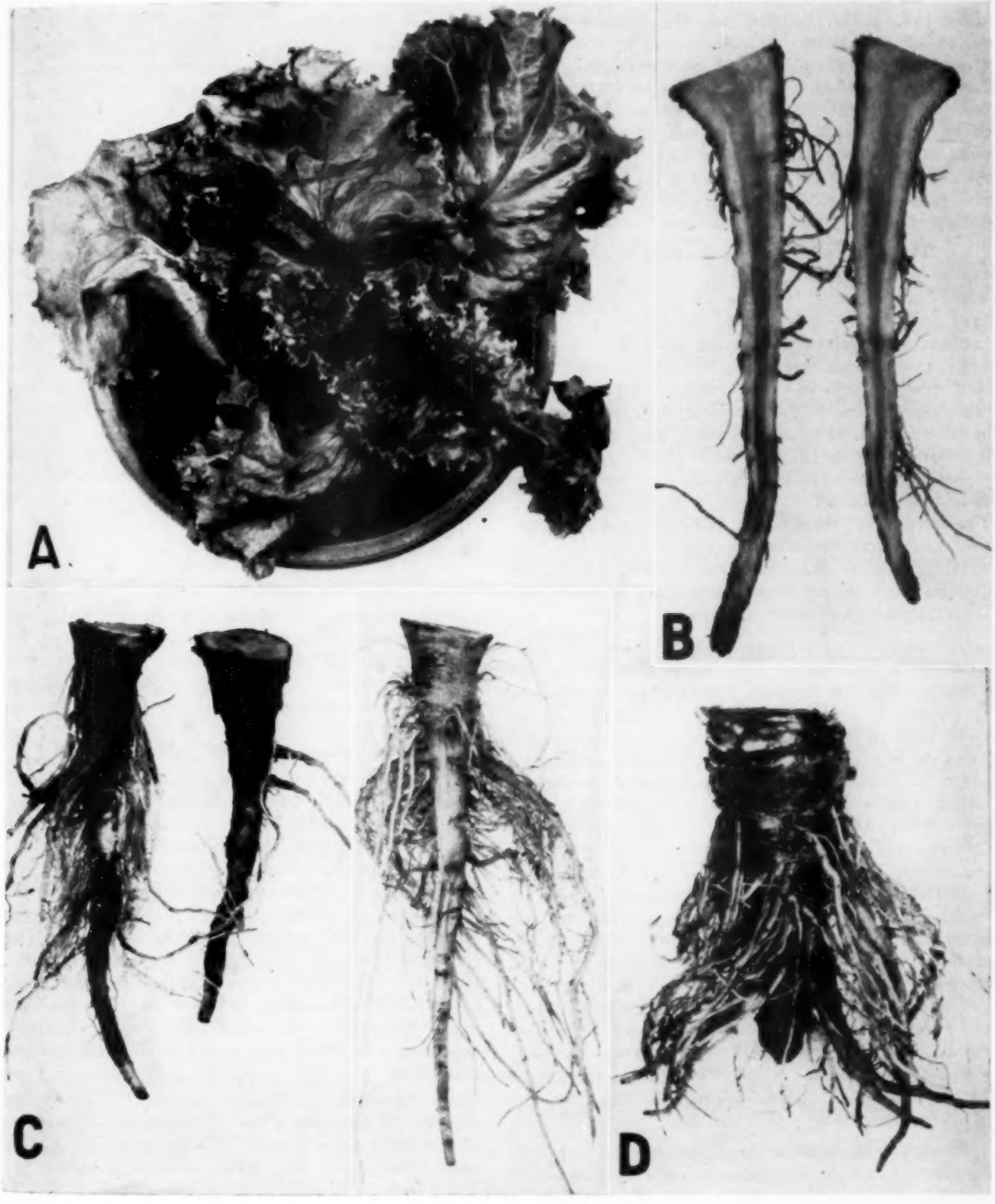

FIG. 1. Injury of lettuce caused by toxic concentrations of fertilizer.—A) Wilting of outer frame leaves induced by the application of chicken manure.—B) Discoloration of central xylem core of root induced by application of concentrated ammoniated ammonium nitrate solution.—C) External browning and corking of root surface induced by chicken manure applied very close to roots (2 roots on left). On the right is a normal root (untreated) from the same experiment.—D) Field specimen showing injury of lateral roots and the tap root.

may become necrotic.

The above ground symptoms are always associated with damage of the root system. Small rootlets, larger lateral roots, and sometimes the tap root may be killed (Fig. 1, D). In most cases the larger roots are not killed completely but exhibit various degrees of yellowing or browning, rifting, and corking. These may occur in isolated portions of the root or may involve practically the whole root system (Fig. 1, C). Often associated with the external damage of the roots is a yellow, red, or brown discoloration of the central xylem core of the root (Fig. 1, B). In cases of severe damage the xylem tissues may collapse, resulting in a cavity that may extend into the crown.

EXPERIMENTAL RESULTS.—*Isolation and inoculation experiments.*—Isolations from injured lettuce roots collected from several locations in the Salinas Valley have given inconsistent results. In most cases, no organisms were isolated from roots showing only slight damage. In some cases, dilution-plate cultures made from severely injured roots yielded several bacterial organisms. Three of these unidentified organisms that have been isolated most frequently were used for the inoculation, by means of needle punctures, of roots of healthy lettuce plants growing in steam-sterilized soil in the greenhouse, but no root damage resulted. The inconsistent results from isolations and the negative results from inoculations indicated that the bacterial organisms that were occasionally present in damaged lettuce roots were secondary invaders following injury from some other cause.

Greenhouse fertilizer application trials.—Experiments were made in the greenhouse with several different nitrogenous fertilizer materials and methods of application to determine the relative toxicity of the compounds to lettuce roots. The variety Great Lakes was used in all tests. Four materials, ammonium sulphate, calcium nitrate, liquid ammoniated ammonium nitrate (containing 28 per cent ammoniacal and 12 per cent nitrate N), and urea were applied as dilute solutions (10 mg per ml) to 1-month-old plants growing in 6-in. pots of steam-sterilized Yolo loam soil. Calculations of the amount of each material required for the different rates of application were based on conversion figures supplied by Smith (21). Each material was applied to 3 groups of 5 pots each at rates of 80, 160, and 320 lb., respectively, of nitrogen per acre. The requisite amounts of the respective solutions were poured on the surface of the soil. One month later the plants were removed from the pots, and the main root was sliced lengthwise and examined for damage. No apparent injury resulted from any of the treatments.

In another greenhouse trial, the same nitrogenous fertilizer materials were applied at the rates of 50 and 100 lb. per acre in the dry or undiluted form to the roots in the bottom of the pots. This was done by upending the pots, dumping out the mass of soil and plant roots, applying the fertilizer to the exposed plant roots, and returning the soil and plants to the pots.

TABLE 1.—*The relative toxicity of fertilizer materials applied at various rates as indicated by root damage of lettuce in the greenhouse*

Material and amount applied per acre	Number of plants of 5 classed according to extent of injury of central xylem core			
	None	Slight	Moderate	Severe
Ammonium sulphate				
50 lb. N	4	1		
100 lb. N	2	2	1	
Urea				
50 lb. N	3	2		
100 lb. N	3	2		
Sodium nitrite				
50 lb. N		5		
100 lb. N			3	2
Calcium nitrate				
50 lb. N	4	1		
100 lb. N	4	1		
Ammoniated ammonium nitrate solution				
50 lb. N		1	1	3
100 lb. N				5
Concentrated phosphoric acid				
50 lb. P_2O_5		1	3	1
100 lb. P_2O_5		1	3	1
Chicken manure				
2 tons	1	2	2	
4 tons		1	4	
8 tons			3	2
16 tons				5
Untreated check	5			

The plants were examined for root injury 6 days later; all treatments showed severe injury and death of rootlets that had been in direct contact with the fertilizer materials. The ammoniated ammonium nitrate solution also caused pronounced discoloration and injury of the xylem core of the main root at rates of both 50 and 100 lb. per acre[2].

The above experiment was repeated with the same 4 materials and, in addition, sodium nitrite and concentrated phosphoric acid. Dried chicken manure that had been ground into a powder also was applied in a similar manner, but at higher rates of 2, 4, 8, and 16 tons per acre. Examination of roots to determine the degree of internal damage was made 12 days after the materials were applied (Table 1). The data from the test involving manure are not comparable with other data presented in Table 1 because of the difference in rates of application. They are included in the table, however, because the damage was very similar in symptomatology to that induced by the other materials.

All materials caused some damage when applied directly to exposed roots. Ammoniated ammonium nitrate and sodium nitrite, however, were considerably more toxic than was urea, ammonium sulfate, or

[2] Plants with damaged roots showed above-ground symptoms similar to those previously described, however, the root symptoms were easier to classify according to severity of damage and therefore were used to obtain the data presented herein.

calcium nitrate. Phosphoric acid induced a similar-appearing breakdown of the internal root tissues and, in addition, caused a necrotic flecking of the older leaves.

Field fertilizer application experiments. — Eleven separate field trials were conducted near Salinas, California, to study the relative toxicity of 5 commercial nitrogenous fertilizer materials commonly used on lettuce in that area. The soils varied in texture from sandy loam to clay. The pH ranged from 6.2 to 8.0, but only 2 of them were below pH 7.0. Plots were located in fields where random sampling of plants indicated that little or no injury had resulted from previous fertilizer applications. The materials were applied at several different rates (expressed as pounds of N per acre) to plants about ½ grown in a way that simulated a commercial side-dress application. A 4- to 5-in.-deep furrow was plowed out with a wheel hoe along the shoulder of the lettuce bed, and the material being tested was then distributed as evenly as possible along 40 ft. of the furrow by hand or from a graduated burette (for the liquid formulations). The material was then covered with soil by filling in the furrow. Observations for internal root injury were made at 7- to 43-day intervals after application. Although the amount and severity of damage resulting from the application of different materials varied from trial to trial, the relative toxicity of the materials was fairly constant. A representative sample of the data presented in Table 2 agrees closely with the results presented in Table 1 and shows that ammoniated ammonium nitrate and aqua ammonia are much more damaging than the other materials. These 2 materials are similar in that both contain free ammonia. Ammonium sulfate and calcium nitrate were relatively nontoxic, but both caused some typical root damage at the higher rates, especially when 43 days had elapsed since application. The increase in amount of damage resulting from increased length of time after application, however, was most striking with urea. One explanation of this phenomenon might be that urea *per se* is relatively nontoxic, but that the toxicity is due to breakdown products from urea produced by microbial or chemical action in the soil.

The data presented thus far indicate that fertilizer materials that contain free ammonia, or other materials that are highly acidic, are more toxic than the neutral salts. To verify this indication, 2 field tests were made in which 5 ml each of different water dilutions of 5 fertilizer materials were injected with a hypodermic syringe directly into the root zone of 20 plants. The

TABLE 2.—*The toxicity to lettuce plants of nitrogenous fertilizers applied at different rates as a side-dressing under field conditions*

Material	Days from application to observation	Lb. N/acre	No. plants in sample	Percentage of roots in indicated severity classes			
				None	Slight	Moderate	Severe
Untreated check	21		64	98.4	1.6	0.0	0.0
Untreated check	43		88	98.8	1.2	0.0	0.0
Ammonium sulphate	21	40	59	100.0	0.0	0.0	0.0
		80	65	93.8	6.2	0.0	0.0
		160	60	95.0	3.3	1.7	0.0
Ammonium sulphate	43	80	71	76.1	23.9	0.0	0.0
		160	68	57.4	35.3	7.3	0.0
		320	56	50.0	32.1	12.5	5.4
Calcium nitrate	21	40	57	100.0	0.0	0.0	0.0
		80	65	100.0	0.0	0.0	0.0
		160	62	90.3	6.5	3.2	0.0
Calcium nitrate	43	80	65	76.9	23.1	0.0	0.0
		160	69	50.7	46.4	2.9	0.0
		320	58	32.8	37.9	19.0	10.3
Urea	21	40	57	98.2	1.8	0.0	0.0
		80	52	94.2	5.8	0.0	0.0
		160	62	93.5	4.9	1.6	0.0
Urea	43	80	75	72.0	21.3	6.7	0.0
		160	66	39.4	34.8	16.7	9.1
		320	24	25.0	0.0	29.2	45.8
Liquid ammoniated ammonium nitrate solution	7	80	61	70.5	4.9	8.2	16.4
		160	64	26.6	12.5	20.3	40.6
		320	51	13.7	21.6	11.8	52.9
Liquid ammoniated ammonium nitrate solution	21	40	54	74.1	5.6	7.3	13.0
		80	57	59.6	7.0	10.6	22.8
		160	51	54.9	2.0	13.7	29.4
Liquid ammoniated ammonium nitrate solution	43	80	70	85.7	0.0	2.9	11.4
		160	62	67.7	1.6	6.5	24.2
		320	60	55.0	5.0	6.7	33.3
Aqua ammonia solution	10	80	68	86.8	8.8	2.9	1.5
		160	51	49.0	17.6	11.8	21.6
		320	46	30.4	8.7	6.5	54.4

TABLE 3.—*Relative toxicity of different concentrations of fertilizer solutions to lettuce roots*[a]

Material before dilution	Concentration as applied[a] (percentage N or P_2O_5)	Number of plants of 20 classified according to extent of injury of central xylem core[b]			
		None	Slight	Moderate	Severe
Control no treatment		20	0	0	0
Aqua ammonia	20.0	0	0	1	19
(20 per cent N)	10.0	0	0	1	19
	5.0	0	1	8	11
	2.5	3	5	9	3
Ammoniated ammonium nitrate solution (40 per cent N)	40.0	0	0	0	20
	20.0	0	0	3	17
	10.0	0	2	0	18
	5.0	12	6	0	2
	2.5	15	3	2	0
Ammonium nitrate solution (20 per cent N)	20.0	18	2	0	0
	10.0	18	2	0	0
	5.0	20	0	0	0
	2.5	20	0	0	0
Nitric acid (15.5 per cent N)	15.5	0	0	3	17
	5.0	2	2	6	10
	2.5	6	14	0	0
Phosphoric acid (54 per cent P_2O_5)	54.0	0	2	5	13
	27.0	1	4	10	5
	10.8	8	6	6	0
	6.75	14	4	2	0

[a] Five ml of each solution under test was applied with a hypodermic syringe at a depth of 7 in. directly into the root zones of 20 plants.

[b] Examinations of the roots to determine extent of injury of the central xylem core were made 5 days after the materials were applied.

results, which are an average of 2 trials, show that aqua ammonia is slightly more toxic than ammoniated ammonium nitrate at the higher dilutions (Table 3). Ammonium nitrate solution, which contains no free ammonia but contains as much nitrogen as the aqua ammonia formulation (20 per cent), caused very little damage even when applied in the concentrated form. This is further evidence that the toxicity of aqua ammonia and of the 40 per cent nitrogen formulation is due largely to free ammonia.

The tests of nitric and phosphoric acid indicate that both materials are toxic; however, they lost more of the toxicity at lower dilutions than did aqua ammonia.

As a further test to determine whether or not the toxicity of aqua ammonia is due to free ammonia, 100-ml portions of aqua ammonia containing 5 per cent nitrogen were treated with different amounts of concentrated nitric acid and compared with untreated aqua ammonia of the same concentration by injecting 5 ml of each formulation into the root zone of 20 plants as previously described. The data from this experiment show that if enough nitric acid was added to partially or completely neutralize the free ammonia, the toxicity was greatly reduced (Table 4). Similar results were obtained by the addition of phosphoric acid, and it is likely that the addition of any acid that would result in the formation of a highly ionized ammonium salt would give similar results.

The relative toxicities of monosodium phosphate, sodium chloride, sodium sulfate, sodium nitrate, sodium nitrite, and magnesium sulfate were compared by injecting 5 ml of a saturated solution of each salt into the soil of the root zones of 2 20-plant groups. The only material that caused any discoloration of the stele was sodium nitrite.

Although the foregoing results strongly indicate that free ammonia is the toxic component of aqua ammonia, there was a possibility that high pH, *per se*, might be the actual cause of root injury. To test this possibility, 2.0, 1.0, 0.1, and 0.01 N NaOH solutions were prepared and 5 ml of each injected into the soil of the root zones of 4 20-plant groups as described previously. Although injection of these solutions resulted in injury and death of the roots that were directly contacted, there was no evident internal root damage or discoloration. Apparently, sodium hydroxide was not absorbed and translocated as were the materials that caused the internal root damage.

DISCUSSION.—The similarity in symptoms produced by the fertilizer materials containing free ammonia and those produced by animal manures indicates that the injury induced by manure is due to free ammonia formed during its decomposition. Likewise the similarity of symptoms produced experimentally by the addition of these fertilizer materials in close proximity to the roots with the symptoms of the previously described lettuce diseases (1, 3, 4, 5, 8, 11, 13, 20, 22) suggests that all of these diseases may have the same basic cause, viz. damage of the root system by ammonia or other toxic nitrogenous materials such as nitrite.

Brown's description (1) of the effect of raw compost in increasing the incidence and severity of the South Carolina lettuce disease strongly indicates that toxins produced during decomposition of the compost were

TABLE 4.—*Toxicity to lettuce roots of aqua ammonia (5 per cent N_2 solution) and of various mixtures of nitric acid and aqua ammonia*[a]

Material or mixture applied	pH of formulation	Number of plants out of 20 classified according to extent of injury of central xylem core of roots			
		None	Slight	Moderate	Severe
Control no treatment		20	0	0	0
Aqua ammonia (5 per cent N)	11.7	0	1	8	11
100 ml aqua ammonia + 5 ml conc. nitric acid	9.5	0	2	6	12
100 ml aqua ammonia + 20 ml conc. nitric acid	8.5	18	2	0	0
100 ml aqua ammonia + 25 ml conc. nitric acid	1.5	19	1	0	0

[a] Five ml of material was applied with a hypodermic syringe at a depth of 7 in. directly into root zone.

the actual cause rather than the bacterial organism (*Xanthomas vitians* (Brown) Starr & Weiss) that she described. The same is true also of the rosette disease of lettuce described by Thomas (22). His interpretation that *Pseudomonas rhizoctonia* (Thomas) Burk. was the cause of the disease is questionable, even though typical root damage resulted when healthy plants were inoculated by pouring bouillon cultures of the organism around the roots. He reported that the organism produced free ammonia quite strongly in bouillon cultures. In view of the results presented herein, one might expect that *P. rhizoctonia* or any other organism capable of producing large amounts of free ammonia by the breakdown of a substrate rich in organic nitrogen could produce the same type of damage to roots inoculated in this manner.

Several nitrogenous materials have been suggested as, and in some cases have been proved capable of, causing damage to plants. Ramsey and Butler (17) described ammonia-gas injury of onions, peaches, apples, pears, and bananas in storage. Russell and Petherbridge (19) reported that as little as 10 ppm of N as free ammonia retarded the germination of turnip seed in sand culture, and 100 ppm completely prevented germination. Willis and Piland (29) described injury in the form of death of the tap root of cotton seedlings following the application of a mixture of potassium nitrate, potassium chloride, and diammonium phosphate. They concluded that the free ammonia formed by the hydrolysis of diammonium phosphate was the most toxic component of the fertilizer. Lorenz et al (10) reported root injury and reduction of yields of spinach, radish, peas, cabbage, lettuce, and onions following the application of aqua ammonia or of anhydrous ammonia. Ammonium sulfate, in contrast, produced no noticeable injury and increased yields in comparison with the unfertilized check plots. They pointed out that aqua ammonia is composed of ammonia dissolved in water and contains comparatively few ammonium ions, whereas ammonium sulfate can be completely ionized. They suggested that the toxicity shown by aqua ammonia or by anhydrous ammonia might be due to the presence of free ammonia that might be more toxic than the ammonium ion.

Several reports of injury to plants from organic sources of nitrogen also have been made. Willis and Rankin (30) reported injury and death of cotton seedling roots resulting from the application of as little as 17 lb. of N to the acre in the form of cottonseed meal. They postulated that this injury resulted from the formation of a toxic concentration of free ammonia by soil microorganisms. Tiedjens (24) claimed that the injury to cotton roots reported by Willis and Rankin (30) was caused by some factor or factors other than ammonia injury. In support of this view, he reported the successful growing of various plants with nitrogen supplied as ammonium sulfate or as ammonium hydroxide at pH values of 7.0 to 8.8. Willis and Rankin (31), in defense of their previous report (30), stated that Tiedjens probably had reduced the amount of ammonium hydroxide to a low level by lowering the pH of the nutrient solution to pH 8.0. Tidmore (23) repeated Tiedjen's work and showed that the acid phosphate content of the nutrient solutions used by Tiedjen was high enough to neutralize the ammonium hydroxide. When weaker nutrient solutions were supplied with nitrogen as ammonium hydroxide, the plants were injured.

Lipman (9) proposed the theory that exanthema of lemons might be caused by ammonia injury. Some of the conditions listed by Fawcett (7) as favoring exanthema lend support to this theory. For example, the disease is more likely to occur near stables, outhouses, cesspools, and poultry yards, where the roots feed in manures, or when dead animals or other offal is buried near the trees.

Nelson (14) described a disease of forcing cucumbers (characterized by increased hydrostatic pressure, severe waterlogging of the leaf tissues, bronzing of the leaf veins, and a generally chlorotic and unthrifty condition of the plant) that was associated with the use of excessive amounts of chicken manure.

Raleigh (16) reported that cow or horse urine applied with complete nutrient solutions to young carrots growing in sand caused marked branching of the roots. Urea, ammonium hydroxide, and ammonium carbonate also caused a branching of the roots that could not be distinguished from that caused by urine.

Nitrites also have been found to be toxic to plants in relatively low concentrations; however, several reports indicate that damage from nitrites is most severe under low soil pH conditions. For example, Mevius and Dikussar (12) reported that corn grown at pH 7.0 could tolerate 200 ppm of N as nitrite, but at pH 4.0, 2 ppm was toxic.

These reports lend support to the evidence presented herein that certain nitrogenous materials are capable of causing damage to plants, but in order to understand better the several factors that influence this phenomenon one must review some of the information concerning the nitrogen metabolism of plants.

Most of the evidence in the many papers on nitrogen metabolism of plants reviewed by Nightingale (15) indicates that neither nitrite nor ammonia nitrogen can accumulate in plants without causing damage. Nitrate nitrogen, in contrast, may be stored in considerable quantities by most plants without causing injury.

There is considerable evidence that most plants detoxify excess ammonia by converting it into the amines, asparagine, or glutamine (26, 27).

Nitrite is reduced rapidly to the ammonia form after absorption by plants and is then converted into organic nitrogen compounds in the same manner as ammonia nitrogen that was absorbed as such directly from the soil (15).

Toxicity of nitrite and ammonia nitrogen probably is dependent upon the relative rates of absorption and utilization or detoxification within the plant. Tiedjens and Robbins (25) reported that nitrate nitrogen is absorbed and assimilated most rapidly

from acid media, whereas ammonia nitrogen is absorbed and assimilated most rapidly from alkaline media. If assimilation or detoxification of ammonia or nitrite keeps pace with absorption so that no accumulation occurs, injury is prevented. However, if ammonia accumulates because of slower assimilation rates at low pH levels or because of too rapid absorption at high pH levels, injury to the plant may result. Lack of carbohydrates, which are necessary for the detoxification of ammonia by conversion to amines, may also result in damage from ammonia (2). Potassium deficiency also has been shown to prevent assimiliation of ammonia, thereby resulting in accumulation of toxic concentrations of ammonia (28). When all of these factors that affect ammonium toxicity are considered, they tend to explain why some reports indicate that ammonium nitrogen is toxic in lowest concentration at very low pH levels, others indicate that the toxicity is due to free ammonia (which can exist only at high pH levels), and still others indicate that ammonia is not toxic at all if abundant carbohydrates are present in the plant.

The extent of damage resulting from ammonia or nitrite derived from decomposing nitrogenous organic matter probably is influenced to a large extent by soil environmental conditions, especially temperature and aeration. The literature concerning the effect of soil aeration on the accumulation of ammonia as opposed to nitrification has been reviewed and summarized by Russell (18) as follows. The decomposition processes leading to the production of ammonia can be performed by a large number of organisms, some of which can function under anaerobic conditions. In contrast, the bacterial organisms that oxidize ammonia to nitrites and nitrates are aerobic. Therefore, if free oxygen is lacking or is in short supply, ammonia is not oxidized but accumulates in the soil.

The effect of temperature and moisture upon nitrification was summarized by Ensminger and Pearson (6) as follows: "It is a generally accepted fact that the greatest accumulation of nitrates takes place during the summer months and the least during winter months. These seasonal variations in nitrates are closely associated with such climatic factors as moisture and temperature."

These reports tend to explain why the disorder described herein is more likely to occur in cold, waterlogged soils.

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OAK WILT DEVELOPMENT IN BUR OAKS[1]

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SUMMARY

Oak wilt development, host-parasite relationships, and symptom expression in bur oaks inoculated with *Endoconidiophora fagacearum* Bretz were studied and compared to those in infected pin oaks. Infection of bur oaks by standard inoculation techniques was difficult. An effective method for inoculation was developed. Small paper pockets were affixed to the trunk and filled with spore suspensions. Chisel cuts were made through the bark into the sapwood below the level of the suspension. Following infection, the spread of the fungus in bur oaks generally was limited to only part of the circumference of the trunk and associated branches. Tyloses, which obstructed water conduction through the affected xylem, developed in the infected vessels of the outer annual ring 2–5 days before foliage symptoms appeared. Only those branches connected to that part of the trunk with plugged vascular tissue developed wilt. Thus, the distributions of the fungus, of vascular plugging, and of foliage wilt were intimately associated and generally were limited. Bur oaks rarely were killed during the first year of infection. As long as a sufficient portion of the vascular system of bur oaks remained unaffected, complete new annual rings were laid down each year. Symptoms recurred on 54 per cent of the trees examined. This recurrence of symptoms resulted from the invasion of the new annual ring by the fungus and the subsequent induction of plugging within its vessels. Living infected trees with recurrent symptoms continued to lay down new annual rings each year until the fungus had spread through the entire crown, inducing plugging of the vessels of the current ring throughout its circumference and, in effect, girdling the tree with plugged vascular tissue. The failure of some bur oaks to show symptoms in years following initial infection resulted from the failure of the new annual rings to become infected from the old. In such trees, new healthy rings were laid down each year, finally burying the infection within the wood. Beetle galleries, presumably made by *Agrilus bilineatus* Web., in the superficial layers of wood of infected branches were commonly associated with the recurrence of symptoms. Isolations from 25 bur oaks that had failed to show recurring symptoms in years following initial infection yielded the oak wilt fungus in only 3 cases. The production of fruiting mats beneath the bark was not observed in bur oaks. Root grafting was infrequent among bur oaks. Tree-to-tree spread of the disease among bur oaks seldom was observed. Trunk and root-collar inoculations were more effective than inoculations through cut branches or roots. Incubation periods were longer than usual in trees inoculated prior to bud-break and in trees inoculated with small numbers of spores. Symptoms were more extensive on trees inoculated with large numbers of spores and on trees inoculated in more than one place around the trunk. Major differences in the wilt syndrome in bur and pin oaks were attributed to the more rapid spread and corresponding general distribution of the fungus through pin oak trees as compared to the generally slow spread and corresponding limited distribution of the fungus through bur oaks.

Oak wilt symptoms on red, black, pin, and other members of the red oak group differ from those on white, bur, and other members of the white oak group (6, 7, 10, 21). In Wisconsin, members of the red oak group infected with the oak wilt fungus, *Endoconidiophora fagacearum* Bretz, generally showed rapid and complete wilting of the entire crown, followed shortly by the death of the aerial portions of the tree. In contrast, infected bur and white oaks usually showed symptoms on scattered branches only. Branch dieback and stagheading increased in cases where the disease progressed from year to year (10). Moreover, in mixed stands, members of the red oak group were killed in large "pockets"; whereas members of the white oak group usually escaped infection and remained as scattered trees (18).

Surveys of local areas in Iowa showed that the percentage of bur oaks killed was much smaller than the percentage of red oaks killed (21). Brinkman (4) studied oak mortality on a 38.6-acre tract of unmanaged timber in Iowa. Despite the fact that white oaks predominated, only 0.4 per cent of them were killed; whereas 11.3 per cent of the red oaks and 13.5 per cent of the northern pin oaks were killed. Such observations suggested important differences in disease development between the 2 oak groups.

The spread of the fungus through the tree appeared to be slower in white oaks than in red oaks (6). Pruning of symptom-bearing branches was reported to prevent further wilt development in some white oak trees but not in others (7). Young (21) observed the recovery of 1 white oak following pruning. No further symptoms appeared, and the fungus could no longer be isolated from the tree.

The purposes of the present investigations were 1) to determine host-parasite relationships and factors affecting wilt development in bur oaks, and 2) to compare oak wilt development in bur oaks with that in northern pin oaks. A preliminary statement has been published (17).

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MATERIALS AND METHODS.—Oak trees used in these studies were scattered through mixed second-growth stands of northern pin oak (*Quercus ellipsoidalis* Hill), jack pine (*Pinus banksiana* Lamb.), and bur oak (*Quercus macrocarpa* Michx.) growing on Plainfield sand in central Wisconsin. Unless otherwise indicated, the trees were from 2 to 8 in. diameter at breast height.

All inoculations were made with concentrated conidial suspensions prepared from 2- to 4-week-old cultures grown on potato-dextrose agar.

Four methods of inoculation were employed. The injection-ax method involved punching a single hole through the bark into the sapwood of the root collar or buttress roots with an injection ax (from the Council Tool Co., Wananish, North Carolina). The hole was about 1.6 cm in diameter and penetrated 1.5–2.5 cm of sapwood. Three to 5 ml of spore suspension were poured into the hole. The suspension usually was taken up by the tree within a few minutes. Nearly all northern pin oaks so inoculated became infected. However, since frequently less than 50 per cent of the bur oaks inoculated in this way developed wilt, a more effective method was sought.

The paper-cone method involved cutting a paper cone from waterproof insulating paper and affixing it to the trunk in the manner described by Beckman et al (3). In most studies, about 100 ml of spore suspension were poured into the cone, and a single 2-cm-wide chisel cut was made into the sapwood under the suspension. This allowed the rapid uptake of large volumes of spore suspension directly into the sapstream. Nearly all inoculations by this method were effective; however, because considerable time and effort were required, a modification was devised.

With the paper-pocket method, cups were cut from waterproof insulating paper and affixed with asphalt roofing compound to a previously smoothed area of the trunk (Fig. 1). Eight to 10 ml of spore suspension were poured into the cup, and a single 2-cm-wide chisel cut was made into the sapwood under the suspension. This method proved effective and the most convenient for routine inoculations. For certain studies, however, special inoculation methods were necessary.

Branch and root inoculations were made by quickly submerging the freshly cut ends of branches or roots in spore suspension contained in a test tube. Often branches had to be tied down in order to hold the test tube in an upright position. Such branch and root inoculations were quite effective on pin oaks but rarely resulted in infection of bur oaks.

In certain studies, the movement of radioactive rubidium carbonate ($Rb_2{}^{86}CO_3$) in the xylem of healthy and diseased trees was traced. The radioactive solutions were introduced into the tree from paper cones in the manner described by Beckman et al (3). The location of the isotope in the tree was detected with a portable Geiger-type monitor (model 2611, Nuclear Instrument Company, Chicago).

Concentrated aqueous safranin-eosin solutions also were used to trace the movement of liquids in the xylem of healthy and of diseased trees. Several hundred ml of dye solution were introduced into each tree in a manner similar to that used for radioactive solutions. The pathway of dye movement was readily observed from cross sections of the trunk or from the exposed surface of the sapwood.

Cross sections of the trunk, branches, and roots were examined with a dissecting microscope or with a 14× hand lens to determine abnormalities of the host vascular system associated with wilt development.

Sections for detailed histological examination were cut with a sliding microtome either from fresh material or from material fixed in formalin-acetic-alcohol. Sections were stained with a solution of safranin O and fast green in 60 per cent alcohol as described by Gram and Jorgensen (9).

Isolations were made from 8- to 12-in. sections of branches or roots by transferring small chips of sapwood to Petri plates containing acidified potato-dextrose agar or Nutramigen agar (16). Successive chips were cut in a spiral fashion so as to include 2 annual rings of the entire circumference and at least 10 linear cm of the section. Trunk isolations were made from 20- to 25-cm bolts split into convenient widths for handling. Plates were incubated at room temperature and were read after 2, 3, and 4 weeks.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—*External symptoms.*—Foliage symptoms on inoculated bur oaks generally were limited to a few scattered branches. At one extreme, trees showed 1 or 2 wilting spurs of leaves; at the other, the entire crown wilted. Most trees showed wilt symptoms on the leader and on 1 or more branches of only 1 side of the tree. Affected branches commonly died. In those trees where symptoms recurred during succeeding years, more branches became involved, dieback increased, and stagheading resulted. Trunk sprouts developed on many trees. These sprouts were in turn killed back until eventually the aerial portion of the tree died. Of the several hundred trees inoculated over a period of 4 years, only 2 died during the first year of infection. Frequently, trees failed to show symptoms during succeeding years.

Foliage symptoms varied with the season of inoculation. In the spring and early summer, young succulent leaves at first became flaccid and wilted during the day and partially recovered at night. No necrosis or discoloration of the leaves was evident during incipient wilt. After a few days, the leaves wilted permanently, shrivelled, and turned brown. On trees inoculated later in the summer after the leaves had matured, loss of luster, marginal or complete bronzing, drooping, and wrinkling of the leaf blade commonly developed. Many of the affected leaves were shed. On some trees, all the affected leaves were shed, and except for the bare twigs, the trees appeared normal throughout the rest of the summer. On trees inoculated late in the summer, yellow to tan mottling

of the leaves appeared. Such leaves suffered little or no loss in rigidity. Premature defoliation did not occur.

Normal and abnormal host anatomy.—In order to recognize internal abnormalities of infected trees, the vascular system of healthy trees was first studied. At 1- to 2-week intervals from early spring until late summer in both 1953 and 1954, healthy trees were cut and cross sections of the trunk and branches were examined with a hand lens. Before bud-break in bur oaks, only the outermost annual ring (formed during the previous growing season) contained open vessels (Fig. 2). The vessels of all other rings were filled with tyloses. Buds began to break on the majority of trees about mid-May in 1953 and about a week later in 1954. The new annual ring began to form at this time. As the leaves expanded, the vessels of the new ring enlarged. A well-defined ring of large springwood vessels became fully developed at about the time that the leaves were fully expanded (about the second

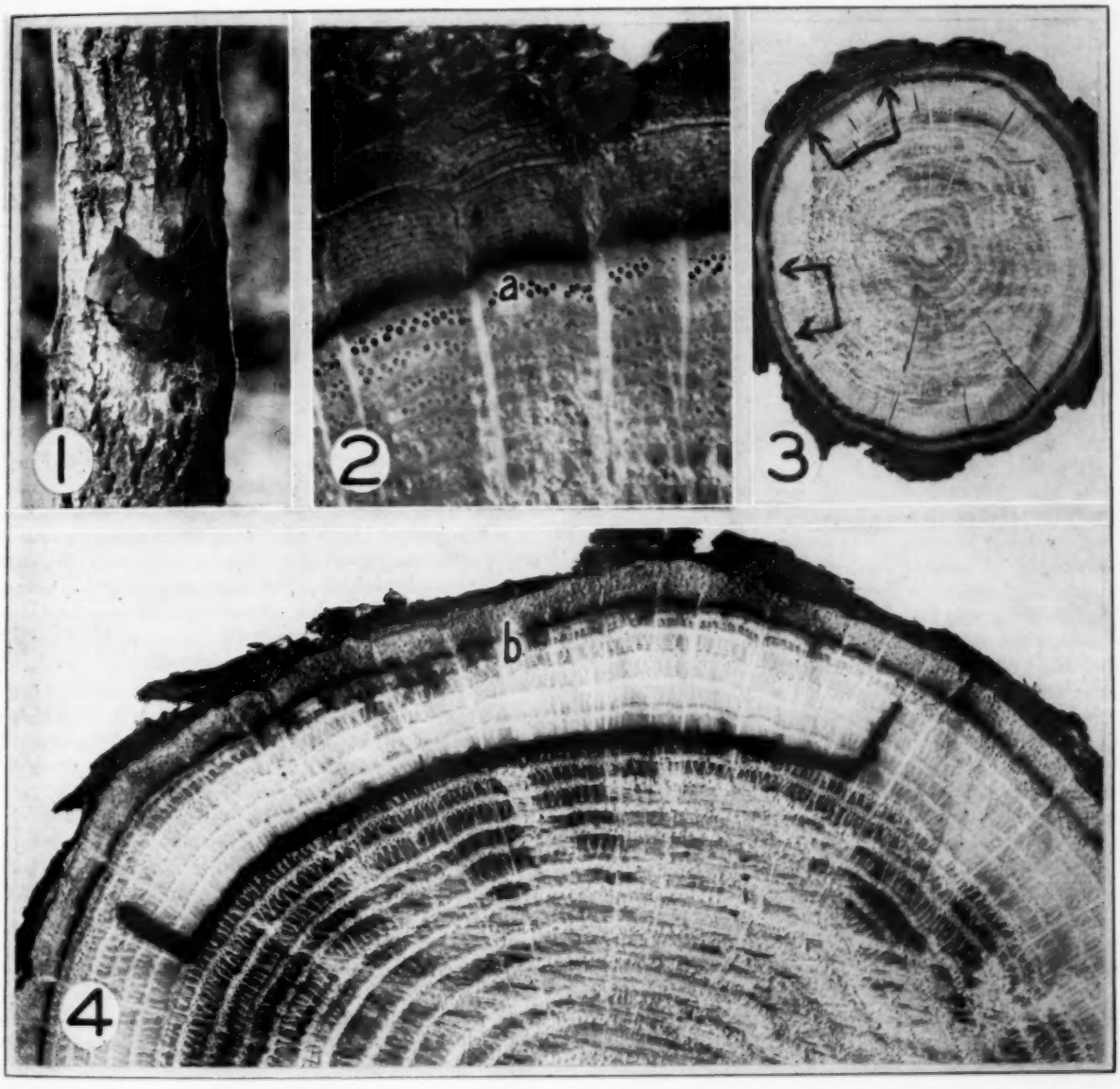

FIG. 1–4. Fig. 1. A paper pocket affixed to the trunk for inoculating the tree with spore suspension.—Fig. 2. A cross section of a normal bur oak trunk showing the single ring of open vessels (a). The vessels of all other rings are occluded with tyloses.—Fig. 3. The pathway of upward movement of dye through the trunk of an infected bur oak. The arrows delimit the plugged arcs of the circumference through which no dye passed. These plugged arcs originated at the 2 points of inoculation in this tree.—Fig. 4. Vascular plugging in the outer ring of the trunk of a bur oak inoculated during the current growing season. The black pencil line delimits the extent of the circumference plugged. Vascular browning (b) has begun to develop in the infected area.

week of June in 1953 and about a week later in 1954). After the new wood was formed, 2 rings with open vessels were present until late June or early July. At that time the vessels of the previous year's ring became filled with tyloses, leaving only 1 ring of open vessels until the next spring. Thus, except for a short period in late June and early July, bur oaks contained only 1 functional ring of vessels.

Cross sections of the trunks and branches of trees inoculated by the injection-ax method were similarly examined shortly after wilt symptoms appeared. These trees, unlike healthy trees, had plugged vessels in the outer annual ring. These plugged vessels were limited to a definite arc of the circumference; the affected areas extended upward and downward from the point of inoculation (Fig. 3). Examination with a hand lens disclosed that the growth of numerous tyloses had plugged the vessels.

Differences between these symptomatic tyloses and normal tyloses were observed with a dissecting microscope. Symptomatic tyloses, found in the outer annual ring, were numerous and packed so tightly in the vessels that they appeared angular as a result of mutual pressure. Normal tyloses, found only in the second and older annual rings, were less numerous and more rounded. In addition, symptomatic tyloses were yellowish *en masse*, as compared to the hyaline appearance of normal tyloses in the sapwood vessels. A slight water-soaking of the tissues frequently was observed around vessels plugged with symptomatic tyloses.

The distribution of symptomatic plugging in the tree and its relation to foliage symptoms were investigated by examining serial cross-sections of the trunk and branches of wilting inoculated trees. The arc of plugged vessels in the outer annual ring extended vertically from the root collar through the point of inoculation to the outer twigs of the crown. The arc of plugged vessels generally extended up 1 side of the tree; in spiral-grained trees, plugging followed the spiral, occasionally diverging as much as 180 degrees from the point of inoculation. Branches connected to the plugged arc of the trunk also contained plugged vessels in the outer ring. Branches that were not connected to the plugged arc had no plugged vessels in the outer annual ring. Below the root collar, plugging was variable. Although occasional plugged vessels were observed in cross sections of the roots below the point of inoculation, no definite plugged arcs were found.

The width of the plugged arc of inoculated trees varied greatly. Of 32 wilting trees that were inoculated through a 2-cm-wide chisel cut, the widths of the plugged arcs at 60 cm above the point of inoculation were 1–5 cm in 8 trees, 6–10 cm in 17 trees, 11–15 cm in 6 trees, and over 16 cm in 1 tree. A small percentage of the crown was wilted in trees with very narrow arcs, and a large percentage of the crown was wilted in trees with very wide arcs. The extent of wilt in the crown was poorly correlated with the extent of the arcs of intermediate widths. The amount of foliage wilt accompanying such arcs appeared to depend in part on the branching habit of the tree and on the number of branches that had vascular connections with the plugged arc of the trunk. An arc 4–6 cm wide sometimes connected with several branches, all of which showed wilt symptoms. In other cases, a similar arc sometimes intersected few, if any, branches. Other internal symptoms appeared later.

Browning of the infected vessels and adjacent tissues was observed late in the season of inoculation or early in the next growing season. The intensity of the browning increased with time. At first, a very light orange-brown color developed. This color gradually deepened into dark brown, similar to the color of the heartwood. Browning usually was limited to the affected annual ring, although the next inner ring occasionally contained a small discolored area. No outward extension of the stain beyond the infected ring was observed. All types of vascular tissues of the ring were brown except the broad rays. In cross sections, browning was continuous in some plugged arcs and discontinuous in others. Vertically, browning was continuous from the smallest affected twigs down to the roots. In the roots, however, vascular discoloration was limited and indistinct.

Over 100 microtome sections from the brown areas of vascular plugging in trunks and branches showed only 2 endoconidia and a few mycelial fragments in the vessels.

Several inoculated trees that failed to show symptoms were examined. An arc of plugged vessels was found in the outer ring of each tree. Unlike plugging in wilting trees, these arcs extended only a few feet beyond the inoculation wound. In addition, tyloses in the plugged vessels were less numerous, and there was no vascular discoloration. Since tyloses often form after wounding (5), the development of tyloses in relation to inoculation wounds was investigated.

Small bur oaks, 4–7 cm in diameter, were wounded near the root collar by punching a hole 1–2 cm into the sapwood with an injection ax. Five trees were wounded on April 23, 1953; 5 more on May 29; and thereafter 5 more every 2 weeks until August 10. The trees were cut 2 weeks after wounding, and the upward extent of wound tylosis formation was measured. Few wound tyloses formed in the spring before the new annual ring was laid down. In trees wounded after the new ring was laid down, tyloses developed in the outer ring and to a limited extent in the second ring (until the second ring became plugged normally). The maximum upward extent of tylosis formation was $2\frac{1}{2}$ m beyond the wound. The widths of the arcs plugged by wound tyloses were only slightly wider than the widths of the associated wounds. Although wound tyloses in mass became yellowish orange, no vascular browning was associated with wound plugging, even after 1 year.

Thus, 3 types of tylosis formation appeared in the sapwood of bur oaks: viz., normal, wound, and symptomatic. Normal tyloses developed spontaneously in

the second annual ring of sapwood but did not occur in the outer ring. Wound tyloses developed in the outer ring following wounding of the sapwood. These tyloses extended upward from the wound for limited distances, above which open vessels were found. No browning of the surrounding tissues occurred with wound-tylosis formation. Symptomatic tyloses formed in the outer ring of infected trees. They occurred from the roots to the current season's twig growth. Yellowish discoloration followed by intense browning of the vessels and surrounding tissues was associated with their formation. Symptomatic tyloses could be distinguished, therefore, by their distribution in the tree and by the associated vascular browning.

Girdling experiments were made to determine whether wounding produced any alteration of the normal appearance of the leaves. Trees in 4 plots, each plot consisting of 5 bur and 5 pin oaks, were girdled through the sapwood of ¼, ½, ¾, or all of the trunk circumference. Bur and pin oaks that were completely girdled showed rapid wilting and browning of the leaves. Two months after girdling, these trees were dead above the girdle. The appearance of the partially girdled trees was indistinguishable from that of nearby untreated trees. These observations indicated that trunk wounds made during inoculation had no effect (on the leaves) with which wilt symptoms might be confused. Apparently both pin and bur oaks have an adequate mechanism for the lateral movement of sap above severe girdles.

Factors influencing external and internal symptom development.—Seasonal variations in the incubation periods of inoculated bur oaks were investigated in 1953 and 1954. Injection-ax inoculations were begun in the spring before bud-break and continued at 2-week intervals throughout the summer. A new group of 10 trees was inoculated at each interval. All trees leafed out normally regardless of the date of inoculation. Symptoms did not appear in the spring until the leaf blades had expanded. Thus, trees inoculated in mid-April and trees inoculated in early June began to show wilt symptoms at the same time. The incubation periods were 8½ weeks and 2½ weeks, respectively, however. On trees inoculated after the leaves had expanded, symptoms usually appeared 12–21 days after inoculation. The date of inoculation did not influence the percentage of successful inoculations or the degree of crown involvement.

The effects of the date of inoculation on the formation of symptomatic tyloses also were investigated. Thirty trees were inoculated by the injection-ax method on April 23, 1954, approximately 4 weeks before bud-break. Each tree was inoculated in 3 places to insure infection. Five of these trees were cut on May 5, and 6 more were cut on May 19. Only the outer (1954) annual ring was open and free of tyloses in these trees. In 5 trees cut on June 8, the 1954 ring and the partially formed 1955 ring were both open. The new 1955 ring was fully developed in the 5 trees cut on June 17. In 4 of these trees, no plugged vessels were found in either the 1954 or 1955 rings. In the 5th tree, an arc of plugged vessels was found in both outer rings. No foliage symptoms had appeared at this time. By June 30, foliage wilt had developed on the remaining trees. Plugged arcs were present in both the 1954 and 1955 rings of the 5 trees cut on that date. Thus, even though trees were inoculated several weeks before bud-break, symptomatic tylosis formation and resultant foliage wilt did not occur until the new annual ring was laid down and the trees were in full leaf.

The effects of spore concentration on the incubation period in bur oaks also were determined. Kuntz et al (15) found that the incubation period in inoculated pin oaks decreased as the number of spores per tree was increased up to 65,000 spores. Further increase in the numbers of spores failed to decrease the incubation period. Similar studies were made on bur oak in August, 1953, by inoculating them with suspensions containing approximately 10^6, 10^5, 10^4, 10^3, 10^2, and 10 spores per ml. These tests were repeated in July, 1954, with suspensions containing 4×10^6 to 4×10 spores per ml in a similar dilution series. The spore concentrations of the original suspensions were determined with a Spencer counting chamber. Succeeding concentrations were prepared by serial dilution. In each test, 10 trees of similar size were inoculated for each spore concentration by introducing a standard volume of suspension, 8 ml per tree, into the xylem by the paper-pocket method. On trees inoculated with 3200 spores or more per tree, symptoms appeared 12–15 days after inoculation. On trees inoculated with 1000 spores or less per tree, symptom expression was delayed by 3 or more days.

The spore concentration apparently also influenced the extent of symptoms. A visual estimate was made of the percentage of the crown showing symptoms. In general, an increase in the concentration of the inoculum resulted in an increase in the percentage of the crown with symptoms. Large variations were encountered, however, in any group of trees inoculated with the same number of spores.

Since previous studies had indicated a correlation between the width of the plugged arc and the extent of symptoms in the crown, quantitative measurements on the extent of vascular plugging in these trees were made. Fifty trees from the 1954 series were cut, and the width of the plugged arcs at 60 cm above the point of inoculation (about 180 cm above the ground) was measured. The average width of the plugged arcs generally increased as the concentration of the inoculum increased (Table 1). Considerable variation among trees inoculated with any 1 spore concentration was found, however. These data suggest that an increase in the number of spores in the inoculum tended to increase both the extent of vascular plugging and, correspondingly, the extent of foliage symptoms.

The effect of the place of inoculation on wilt progression in the trees and on possible control measures

TABLE 1.—*Widths of the plugged arcs in the outer annual ring of bur oaks inoculated with different numbers of spores of the oak wilt fungus*

Tree no.	Width in cm of plugged arc[a] in trees inoculated with indicated number of spores (× 3.2) per tree					
	10^2	10^3	10^4	10^5	10^6	10^7
1	3.4	7.5	10.7	14.5	12.0	28.2
2	4.0	7.0	6.0	18.0	13.0	11.5
3	11.0	20.0	10.0	9.0	7.5	23.5
4	7.0	4.0	14.5	11.5	3.8	5.2
5	8.0	5.0	17.0		4.7	15.5
6	6.0	7.3	7.0		11.0	14.0
7	5.0	7.0	5.9		10.0	14.0
8	8.0	11.0	7.3		9.0	24.5
9	5.0	2.2	14.0		29.9	6.5
10	10.0		9.0			
Average	6.7	7.9	10.1	13.2	11.2	15.8

[a] At 60 cm above the point of inoculation.

was investigated. Kuntz et al (15) demonstrated that following branch inoculations, wilt symptoms appeared first on the inoculated branches of northern pin oaks. Moreover, the fungus was restricted to the inoculated branch for some time before it spread to the rest of the tree. It was often as long as 7 weeks before the entire crown wilted. This was in comparison to 3 weeks in trees inoculated at the root collar. Similar lateral branch inoculations were made with bur oaks by fastening test tubes containing spore suspension over the cut ends of living lateral branches. Two hundred thirty-five trees were inoculated during 4 years. Only 14 of these trees developed wilt symptoms. In pin oaks, on the other hand, such inoculations induced a high percentage of infections (15).

In the present work, isolations were made to determine if the fungus became established in inoculated branches of trees that failed to develop symptoms. One month after inoculations, isolations were made at 20-cm intervals within 60 cm of the point of inoculation on the branches of 20 symptomless trees. The fungus could not be isolated from these branches. Isolations from the inoculated branches of 3 trees that developed symptoms were positive. Apparently the fungus had not become established in the inoculated branches of trees that failed to develop wilt symptoms.

The extent of the symptoms varied on the 14 trees that were successfully inoculated. Since the inoculated branches of some trees had no leaves, and since the times of examination varied, the exact sequence of foliage-wilt development could not be determined for all trees. At the times of examination, 1 tree had developed symptoms involving most of the crown, 8 had developed typical scattered symptoms, and 5 had developed symptoms initially on the inoculated branches only. The inoculated branch of these 5 trees was pruned off at the trunk shortly after symptoms appeared. Cross sections from the bases of the branches were examined with a hand lens. The outer ring of every branch was partially or almost entirely plugged. During the 6 weeks following pruning, further symptoms developed on only 2 of the 5 trees. All 5 trees were then cut, and cross sections of the trunks both above and below the inoculated branches were examined. The 2 trees that had developed further symptoms contained narrow plugged arcs in the outer ring both above and below the level of the inoculated branch. Plugging in the other 3 trees was sparse and indefinite. Since some plugging could have resulted from the pruning wounds, the 5 trees were allowed to lie on the ground in order to determine whether the xylem of the trunk had been invaded. The following spring they were examined for vascular browning. The wood was still fresh and browning was easily observed. The 2 trees that had shown further symptoms and contained plugged arcs in the trunk developed vascular browning. The other 3 trees showed no browning. Probably, at the time the branches were pruned off, the fungus had invaded the trunks of the 2 trees but had failed to invade the trunks of the other 3.

Disease development resulting from root inoculation was considered. A small, lateral root on each of 35 bur oaks was exposed, cut, and immediately submerged in a spore suspension contained in a test tube. Approximately 7 weeks later, 2 of these trees developed scattered foliage wilt. The other 33 trees failed to develop symptoms. The inoculated roots from 8 trees that failed to show wilt symptoms were examined and were found to be plugged with wound tyloses for only 15–30 cm from the point of inoculation. No plugging was found in the remaining length of the roots or in the trunks. In contrast, the inoculated roots of the 2 trees that developed wilt symptoms were generally plugged throughout their length, and the trunks contained plugged arcs in the outer annual ring. Thus plugging in inoculated roots was quite different from that in the roots of trunk- or collar-inoculated trees, where plugging was usually inconspicuous.

Since vascular plugging and foliage wilt resulting from single inoculations were limited, the effects of multiple inoculations were determined. Inoculations by the paper-pocket method were made on 10 trees at 1 place on the trunk, on 10 trees on opposite sides of the trunk, on 10 trees at 3 places spaced around the trunk, and on 7 trees at 4 places spaced around the trunk. All inoculations were made at the same height. In general, the extent of foliage symptoms increased as the number of places of inoculation increased. Three trees in each series were examined for plugging. In each tree, a plugged arc, originating at each point of inoculation, intersected wilting branches. Hence, the number of wilting branches increased as the number of points of inoculation increased.

The relation of vascular plugging to foliage wilt.—The above observations suggested a close correlation between vascular plugging and foliage wilt. In previous tests made on pin oaks at the time of symptom expression, the movement of radioactive solutions and other liquids in the xylem was reduced by 80 to 95 per cent (3). In the present studies, attempts were made to determine if a similar reduction in the movement of materials through the xylem occurred in wilt-

ing bur oaks. Twelve inoculated bur oaks with scattered foliage wilt were treated with radioactive rubidium carbonate solution. The radioactive solutions were taken up through continuous chisel cuts made into the sapwood around the entire trunk circumference. When uptake of the solution was complete (usually within 1 hour), the trees were cut, and the branches were tested for radioactivity. Radioactivity was readily detected in the symptomless branches, but no activity was detected in the wilting branches.

Concentrated aqueous safranin-eosin dye solutions were introduced into 30 trees showing typical scattered foliage wilt to check the results obtained with rubidium. Again, chisel cuts were made into the sapwood around the entire circumference. Within 3 to 5 hours after treatment, most of the symptomless leaves were dyed red, whereas leaves with symptoms were not. The trees were cut, and cross sections of each branch and of the trunk below each branch were examined for dyed xylem. In the trunks of all trees, the dye solution moved freely in the open vessels of the outer annual ring, but none moved through the vessels of the plugged arcs (Fig. 4). The dye solution moved readily into symptomless branches. Vessels from unplugged portions of the trunk xylem entered such branches. The dye solution failed to move into the wilting branches. Vessels from the plugged arc of the trunk entered such branches. On some branches, only part of the leaves showed symptoms. These branches arose partly from the plugged arc and partly from the open portion of the trunk xylem. Both plugged and open vessels were found in the outer ring of such branches.

In contrast, no movement of dye solutions occurred at any place around the circumference of 5 wilting northern pin oaks treated with dye. The vessels around the entire circumference of wilting pin oaks were plugged at the time of symptom expression. In both species, the movement of liquids was effectively blocked by vascular plugging.

If the formation of tyloses and the subsequent plugging of the vessels preceded the development of leaf symptoms, such plugging might have contributed to the development of leaf symptoms. Vascular plugging in inoculated pin oaks has been reported to precede leaf symptoms by as much as 4–5 days (3). The time of tylosis formation in relation to leaf symptoms in bur oaks was investigated in August of 1953 and in both June and July of 1954. In each test, 24 trees were inoculated, each in 3 places, with about 100 ml per tree of a concentrated spore suspension by the paper-cone method. At 2- to 3-day intervals following inoculation, 4 of the trees were cut and serial cross-sections of the trunk and terminal leader were examined for tyloses in the vessels of the outer annual ring. In all 3 tests, wound tyloses were well developed by the sixth day following inoculation. These tyloses filled the vessels of a definite arc of the outer ring above the inoculation wounds. Such plugged arcs gradually disappeared as sections were taken farther from the point of inoculation. At 2.4 m above the point of inoculation, only occasional plugged vessels were observed; at 3.6 m, all the vessels were open. Symptomatic tyloses, however, did not appear in the vessels until the eighth to eleventh day after inoculation.

In the vessels of the outer annual ring of trees cut 8–11 days after inoculation, tyloses were evident at all heights. These tyloses were first visible with a hand lens as round or elongate hyaline protrusions with a diameter about ⅛ to ¼ that of the vessels. In trees cut at 10–13 days, tyloses had grown sufficiently to press against one another and fill the vessel lumina. Still, no leaf symptoms had developed on these trees. Incipient wilt symptoms appeared on the remaining trees 12–15 days after inoculation. Thus, plugging had preceded wilt symptoms by 2–5 days.

Further studies on the relation of vascular plugging to leaf symptoms were made by tracing the movement of concentrated aqueous safranin-eoson solutions in inoculated trees. Twenty-four trees 10 to 15 cm in diameter were inoculated by the paper-cone method. Seven days after inoculation, and at 2-day intervals thereafter until symptoms appeared, 6–8 ft. of the terminal leaders were cut from different sets of 4 trees. The cut ends were immediately submerged in dye solution. These branches were examined 5 hours later. Dye solutions had moved freely in the outer ring of all 4 branches cut at 7 days. Both the outer annual ring and the leaves were deeply dyed. No tyloses were found in the vessels. In terminal branches from trees cut at 9 days, the vessels and leaves were less intensely dyed. Small tyloses were present in most of the vessels. They were too small to fill the vessel lumina, however. Three of the 4 terminal branches from trees cut at 11 days failed to transport the dye through most of the circumference. The fourth terminal branch showed reduced uptake. The vessels of the nonconducting portion of the outer ring of the 3 terminals were filled with tyloses. Tyloses too small to fill the vessel lumina were found in the fourth terminal. Thus, vascular plugging preceded foliage wilt by 3–4 days. Symptoms appeared on the remaining trees at 11–13 days. Beckman et al (3) found that a similar obstruction of water movement in the xylem of pin oaks occurred 3–4 days before leaf symptoms appeared.

These observations support the hypothesis of Beckman et al (3) that foliage symptoms result primarily from lack of water because of the plugging of the water-conducting vessels by tyloses and gums. The mechanism of wilting in bur oaks appeared similar to that in pin oaks.

Distribution of the fungus in wilting trees.—The limited vascular plugging and associated leaf symptoms in bur oaks suggested that the distribution of the fungus in the tree also might be limited. In pin oaks, not only did the vascular plugging and subsequent foliage wilt involve the entire crown, but at the time of symptom expression, the fungus could be iso-

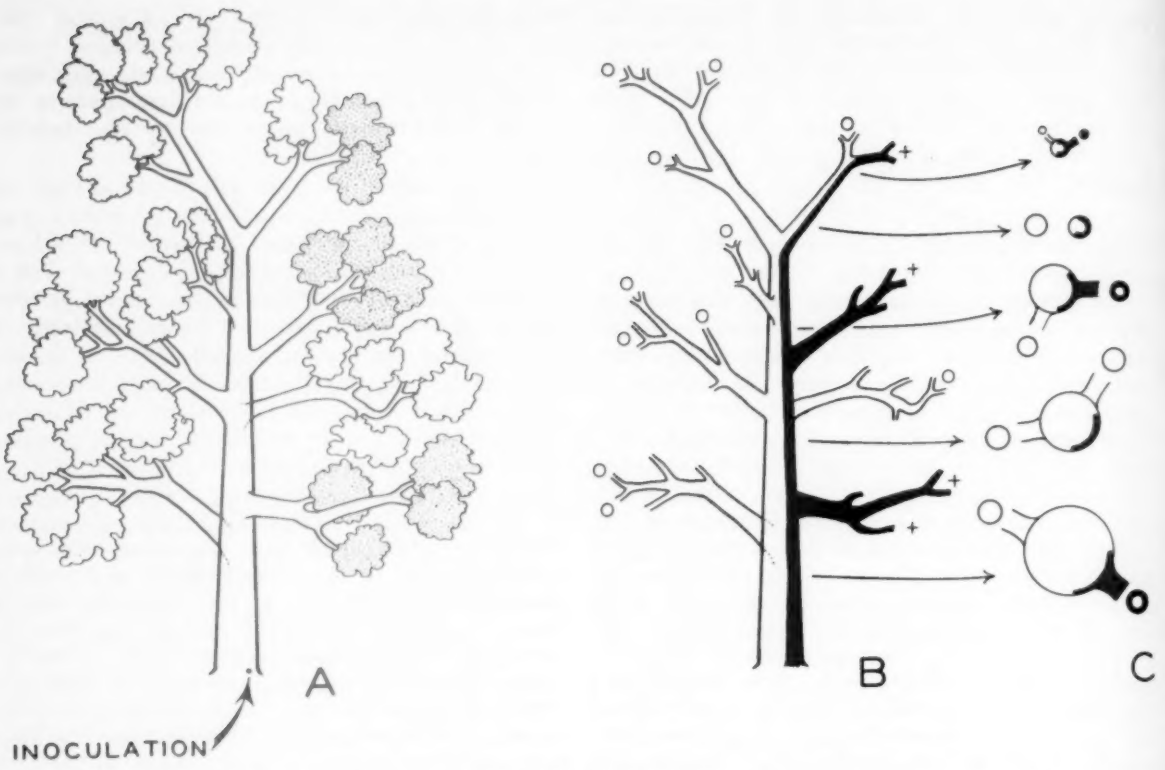

FIG. 5. A) Diagram of the foliage symptoms (indicated by shaded areas) on a representative inoculated bur oak. B) The distribution of vascular plugging (dark area) and the fungus (+) in the same tree. Branches without plugging did not yield the fungus (o). C) Cross sections of the tree showing the relation of plugged infected branches (black) and open, noninfected branches (white) to the plugged arc of the trunk.

lated from practically all parts of the crown (11, 15). The distribution of the fungus in bur oaks was determined in 20 trees with typical scattered foliage wilt resulting from inoculation through a single injection-ax punch hole. Each tree was cut, and serial cross-sections were taken every 90–120 cm up the trunk. Sections also were taken below each branch and from the base of each branch. The position of the plugged arc and the relation of all symptomless and symptom-bearing branches to the plugged arc were diagrammed (Fig. 5). All symptomless branches arose from the open area of the trunk xylem and were free of plugging. Symptom-bearing branches were seated partly or entirely in the plugged arc of the trunk xylem and contained plugged vessels in the outer ring. Samples for isolation were taken from all major branches.

In all 20 trees, isolations from the symptomless branches were negative. Isolations from the symptom-bearing branches (Fig. 5) were generally positive. Isolations from the open areas of the trunk xylem were negative, whereas isolations from the plugged arc of the trunk were generally positive. In all 20 trees, the distribution of the fungus coincided with the distribution of plugging in the outer ring of vessels. Likewise, the occurrence of foliage symptoms coincided with the distribution of plugging. In no case did foliage symptoms appear, nor was the fungus isolated from areas of the crown that did not contain plugged vessels. Thus, unlike disease progression in northern pin oaks, both infection and resulting symptoms were limited in bur oaks.

Differences in the movement of materials through the xylem of bur and of pin oaks that might clarify the observed differences in the distribution of the fungus were investigated. Paper cones were placed on 13 healthy northern pin oaks and on 13 healthy bur oaks. Several hundred ml of dye solution were poured into each cone. A single 2-cm-wide chisel cut was made into the sapwood beneath the surface of the dye. Uptake was rapid in both groups of trees. The trees were cut 5 hours after treatment, and the limits of dye movement were observed from serial cross-sections of the trunk. The width of the dyed arc of sapwood and the percentage of the circumference dyed were recorded. The width of the dyed arc of sapwood was much greater in northern pin oaks than in bur oaks (Table 2). Moreover, as sections were taken farther up the trunk, the width of the dyed arc slowly increased in pin oaks but slowly decreased in bur oaks. Since the tangential movement of dye solutions was greater in pin oaks than in bur oaks, experiments were designed to determine whether the fungus be-

TABLE 2.—*The distribution of dye solution in the xylem of healthy pin and bur oaks treated through a single 2-cm-wide chisel cut*

Height above point of treatment	Width of stain area[a]		Percentage of the circumference stained [a]	
	Pin	Bur	Pin	Bur
cm	cm	cm		
60–120	8.6	3.6	36	15
180–240	9.7	3.4	51	20
300–360	9.8	3.0	58	22
420–480	10.4	2.5	73	24

[a] Each figure is the average of measurements taken from 13 trees.

came distributed in a comparable manner.

The lateral spread of the fungus in the tree was investigated by blocking the direct upward or downward movement of spores through the xylem. Paper pockets were fastened to the trunks of 46 bur oaks and 43 pin oaks. Before inoculation, a notch 10–15 cm wide was cut into the heartwood at 15–30 cm above the pocket on 18 bur oaks and 18 pin oaks, at 15–30 cm below the pocket on 18 bur oaks and 18 pin oaks, and a similar distance both above and below the pocket on 10 bur oaks and 7 pin oaks. Each notch was wide enough to break all direct vascular connection to areas of the tree beyond the notch. Inoculations through single 2-cm-wide chisel cuts were made immediately after the notches were cut. Two of 18 bur oaks and 11 of 18 pin oaks notched above the point of inoculation developed wilt; 15 of 18 bur oaks and 15 of 18 pin oaks notched below developed wilt; and 1 of 10 bur oaks and 5 of 7 pin oaks notched both above and below developed wilt. Isolations made from 3 of the wilted pin oaks notched above the point of inoculation showed that the fungus was present throughout the crown. Similar isolations from the 2 wilted bur oaks notched above showed that the fungus was present in the wilted branches of the crown. The fungus could not be isolated from the crowns of 3 bur oaks and 3 pin oaks that failed to show symptoms. Regardless of the location of the notches, the fungus invaded the crown and induced symptoms in a high percentage of pin oaks. The fungus invaded the crown of a high percentage of bur oaks notched below the point of inoculation, but failed to invade the crown of most bur oaks notched above. These observations indicated that the tangential distribution of the fungus was more rapid in pin oaks than in bur oaks, and that the fungus had to move upward into the crown before wilt symptoms occurred.

Recurrence of symptoms in years following initial infection.—Since bur oaks were rarely killed during the first year of infection, it was possible to study disease development during succeeding years. Of 130 inoculated bur oaks that showed wilt symptoms in 1953, only 70 trees developed symptoms in 1954. Symptoms ranged from 1 or 2 wilted spurs of leaves to almost complete involvement of the entire crown. In 1954, second-year wilt symptoms began to appear about the same time that first-year symptoms appeared on trees inoculated early in the spring of 1954. Of the 70 trees that developed second-year symptoms in 1954, 27 were showing symptoms by June 21–30, 36 more by July 7, and 7 more by August 19. These trees were examined for other host responses.

Vascular plugging associated with second-year wilt was compared to that of first-year wilt. Seventy-four trees that showed typical wilt symptoms during the summer of 1953 were selected for further study. Thirty-six of these trees showed wilt symptoms in 1954. All 74 trees were cut in late summer and trunk cross-sections were examined for plugging and vascular browning. In all cases, the infected area of the 1953 ring was clearly indicated by vascular browning (Fig. 6). In the spring of 1954, normal cambial activity was resumed, and a new annual ring was formed in all trees. This new ring was laid down over both the infected and the noninfected portion of the 1953 ring. In trees with typical scattered wilt during 1953, the new annual ring growth was comparable to that in healthy trees. In trees in which the 1953 symptoms were extensive, the new-ring growth was less and the vessels were smaller.

A plugged arc was found in the new (1954) ring of all 36 trees on which symptoms recurred in 1954. The arcs of the 1953 and 1954 rings were not necessarily in juxtaposition. In many trees, the new arc was offset somewhat, indicating tangential as well as radial movement of the fungus. The 2 arcs always overlapped at some point, however. The new arcs in the 1954 ring varied in width from smaller to much larger than that of the previous year. Frequently there were 2 or more separate smaller arcs in the new ring, which suggested that the fungus had invaded the new ring from 2 or more places along the old (1953) arc. Isolations from the plugged arc of the new ring were positive, indicating actual invasion of the new ring by the fungus. As with first-year infection, dyes and radioactive materials moved freely in the open portions of the new ring but did not move in the plugged arcs or in the branches connected to them.

In the 38 trees that failed to show symptoms in 1954, the new annual ring remained entirely open (Fig. 6). No tyloses were formed in the vessels, and dyes and radioactive materials moved freely through the 1954 ring. Branches that were killed in 1953 did not, of course, take up these solutions. The fungus could not be isolated from the new annual ring. Thus, in trees that failed to show symptoms in 1954, the fungus failed to invade the new annual ring and no vascular plugging or discoloration occurred. Trees that were in the third or fourth year after initial infection were also examined.

Twenty-three trees that had been inoculated and that had shown symptoms in 1952 were cut in the summer of 1954. Six trees showed wilt symptoms in 1953 and in 1954. Vascular browning was evident in the 1952 and 1953 rings, and a plugged arc was found

in the 1954 ring of these trees. Five trees showed symptoms in 1953 but not in 1954. Vascular browning was evident in the 1952 and 1953 rings, but the 1954 ring remained open and unaffected in these trees. Twelve trees failed to show symptoms either in 1953 or 1954. Vascular browning was evident in the 1952 ring. No browning or symptomatic plugging occurred in the 1953 or 1954 rings. Apparently, symptoms developed only in those years in which the fungus invaded the new annual ring and brought about vascular plugging.

Eight trees that had been inoculated and that had shown symptoms in 1951 were cut and examined in 1954. In all 8 trees, new annual rings were laid down in 1952, 1953, and 1954. Five of these trees showed vascular browning in the 1951 ring but no evidence of fungus invasion of the 1952, 1953, or 1954 rings (Fig. 7). These trees showed symptoms in 1951 only. Three trees developed symptoms in all 4 years. In these trees, the 1951, 1952, and 1953 rings contained arcs of vascular browning, and the 1954 ring contained a plugged arc. Again, wilt symptoms occurred only in those years in which the corresponding annual ring showed evidence of fungus invasion.

Terminal growth in 6 trees that showed evidence of infection in only the 1951 ring was also examined. The

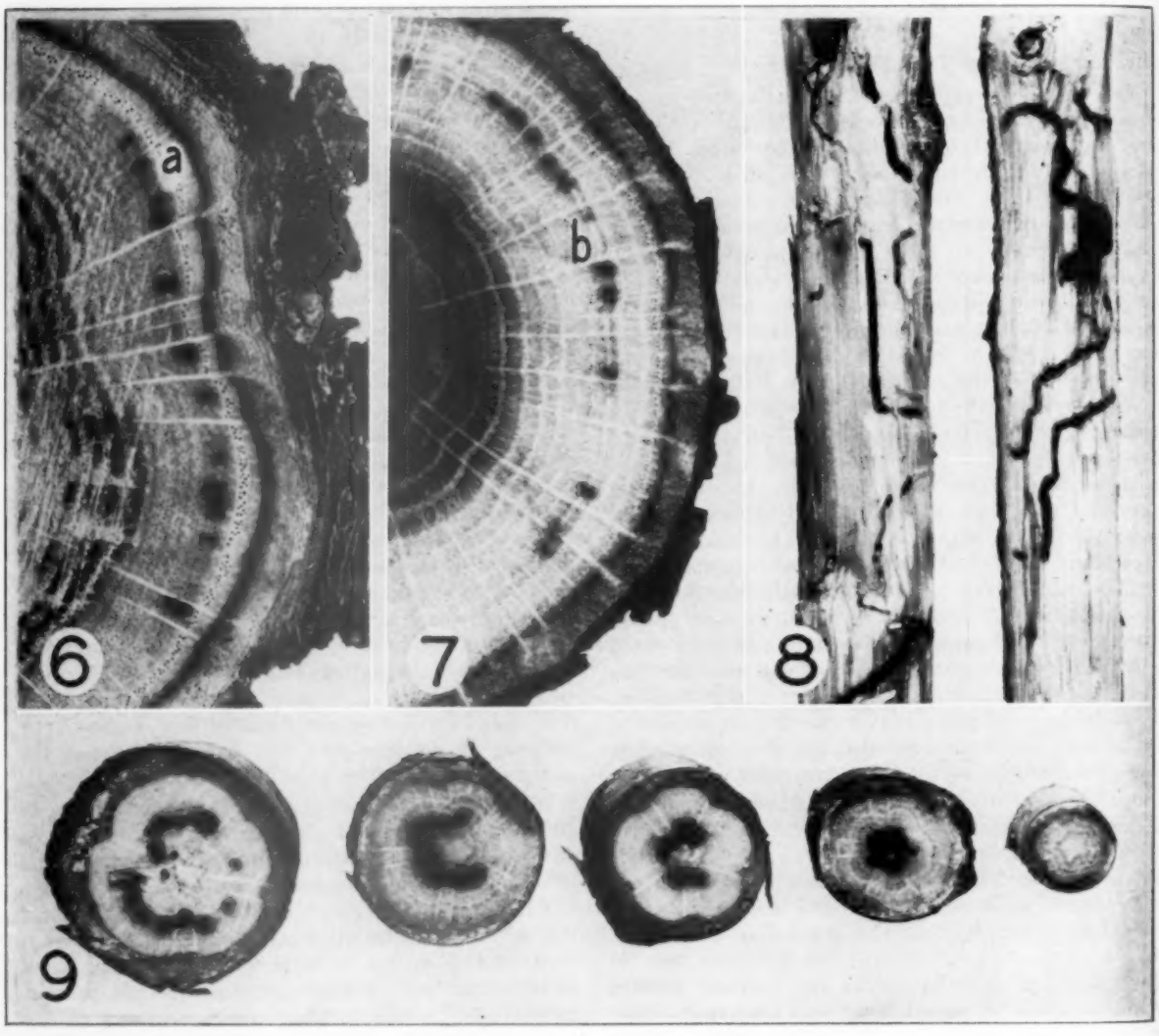

FIG. 6–9. Fig. 6. A cross section of the trunk of a bur oak that developed wilt symptoms in 1953 as a result of inoculation in 1953. Vascular browning in the 1953 ring indicates the area of infection. The 1954 ring (a) remained open in 1954, and no further symptoms appeared.—Fig. 7. A cross section of the trunk of a bur oak that developed symptoms in 1951 as a result of inoculation in 1951. No further symptoms developed in 1952, 1953, or 1954. Vascular browning occurred in the 1951 ring (b), but no vascular browning or symptomatic plugging occurred in the 1952, 1953, or 1954 rings of this tree.—Fig. 8. Galleries of the 2-lined chestnut borer in the branches of a bur oak with second-year symptoms.—Fig. 9. Serial cross sections from the branch of a bur oak that was inoculated in 1951 but failed to show symptoms in succeeding years. Vascular browning occurred in only the 1951 vascular tissue. No vascular browning or symptomatic plugging occurred in the 1952, 1953, or 1954 rings or in the 1952 internode.

year in which a given internode was produced was determined by ring count. The xylem tissues, including the primary xylem, produced in 1951 showed browning. The terminal growth produced in 1952, 1953, and 1954 showed no evidence of fungus invasion (Fig. 9). Thus, new shoot growth appeared to be noninfected in trees that failed to show symptoms in years following the development of initial wilt symptoms.

Trees with and without recurring symptoms were examined to determine whether mycelium was present. Microtome sections of the outer annual rings were prepared from both the trunks and the branches. No mycelium or spores were observed in the vessels or surrounding tissue either in trees that showed apparent recovery or in trees that had recurring symptoms. Other studies were made to determine the condition of the fungus in the host in succeeding years.

The absence of leaf symptoms or evidence of fungus invasion of the outer rings of many trees in years following the appearance of initial wilt symptoms suggested that such trees might have recovered. Isolations were made to determine whether the fungus remained viable in such trees. Before the isolations were made, cross sections of the trunk of each tree sampled were examined to insure that the outer ring was free of plugging. Samples for isolation were taken from live branches that showed vascular browning in 1 or more of the inner annual rings of the sapwood. In this way, originally infected branches could be differentiated from branches that never had been infected. From 2 to 8 branches per tree were sampled. At least 2 sections were taken from each branch; 15 or more chips, including 2–3 outer annual rings, were cut from each section. All plates were kept under observation for at least 1 month. Trees that were actively wilting for the second or third consecutive year were sampled as controls. Of the 35 trees without symptoms, 32 failed to yield the fungus (Table 3). All 11 of the trees with symptoms yielded positive cultures.

Isolations were made from the individual annual rings of 4 trees that showed active wilt for 4 or more summers in succession. Isolations were made from annual rings exposed by splitting trunk sections radially. Positive plates were obtained from the outer and second annual rings. No positive plates were obtained from the deeper rings.

No general characteristics of the trees or of symptom development during the first year of infection could be correlated with the presence or absence of symptoms during succeeding years. Some trees with extensive foliage wilt failed to show symptoms during the second year, whereas some lightly affected trees developed further symptoms. Certain disturbances in trees with second-year symptoms were noted, however.

Beetle galleries were often observed in branch sections taken for isolation from trees with recurring symptoms. Such galleries were not observed in sections taken from trees that failed to show symptoms in the second year. Since there appeared to be some association between the presence of beetle galleries and the occurrence of second-year symptoms, trees with and without second-year symptoms were studied. Twenty-five trees with second-year symptoms and 10 trees without second-year symptoms were examined for evidence of fungus invasion of the current-season's wood. As with previous studies, plugged arcs were found in the outer annual ring of all trees with symptoms. No plugging was found in the outer ring of the 10 trees without symptoms. All major branches were debarked from the tip down to a point where the diameter was about 4 cm. The presence or absence of beetle galleries was recorded. Though galleries lower down in the tree were not recorded with this method, the prevalence of galleries in the upper branchs indicated that the method gave a fairly accurate assay of beetle attack. Galleries were found (Fig. 8) in the branches of 20 of the 25 trees with second-year symptoms. No galleries were found in the branches of the 10 trees without second-year symptoms.

TABLE 3.—*Number of trees from which the oak wilt fungus was recovered in the second and third years following inoculation*

Year inoculated [a]	Symptoms in 1954[b]	No. of trees that gave indicated isolation reaction	
		Positive	Negative
1952	—	0	16
	+	3	0
1953	—	3	16
	+	8	0

[a] All trees showed symptoms the year of inoculation.
[b] — Indicates no symptoms; + indicates symptoms.

The majority of the galleries were limited to the cambium and inner bark. Occasionally, however, a gallery penetrated into the sapwood through 1 or more annual rings. The pattern of the galleries suggested the work of the 2-lined chestnut borer, *Agrilus bilineatus* Web. Since no larvae were present in the galleries of trees that had showed oak wilt symptoms for 2 summers, trees inoculated and showing symptoms during the current summer were examined.

Seven bur oaks inoculated in June 1954 were examined during the following winter. These trees showed wilt symptoms during the summer, and plugged arcs were present in the outer ring. Four trees had beetle galleries in the upper branches. Several larvae of the genus *Agrilus* were found in the galleries. The other 3 trees showed no evidence of beetle attack. These observations suggest that beetle injury may be instrumental in perpetuating the infection in some trees. They also suggest that pruning experiments might help to clarify this relationship.

Natural occurrence and spread.—Natural infection of bur oaks was observed only rarely in forest stands in central Wisconsin but was not uncommon in parks, yards, and other open areas. Three naturally infected bur oaks were examined for tyloses and plugging. Two of these trees were in the first year of wilt. They had plugged arcs in the outer ring of the trunk similar

to those in inoculated trees. The third tree appeared to be in the second year of wilt. In addition to the plugged arc in the outer ring, an arc of vascular browning was found in the second annual ring. No differences in the syndrome of naturally infected and that of inoculated trees were observed.

In large oak wilt "pockets" where pin oaks were killed by progressive tree-to-tree spread, bur oaks usually escaped infection. In 4 years of study involving hundreds of inoculations, no tree-to-tree spread was observed among bur oaks or between pin and bur oaks. It has been shown that root grafts among pin oaks contribute to the rapid tree-to-tree spread of the disease in that species (2, 15). Root grafting among bur oaks was studied by selecting individual trees in dense stands where both bur oaks and northern pin oaks were present. Ninety-five bur oaks 8–35 cm in diameter were treated with sodium arsenite solution through basal cones or through multiple punch holes around the root collar. The arsenite solution was translocated from the treated bur oak to an adjacent bur oak in only 5 cases. In only 2 cases the arsenite solution passed from the treated bur oak to an adjacent nothern pin oak. When these root systems were excavated, functional interspecific grafts were found between the roots of the bur and the northern pin oaks. Thus, root grafting among bur oaks was rare, and grafting between bur and northern pin oak was very rare.

Fruiting mats of the fungus on bur oaks were not observed in central Wisconsin, even when fruiting was abundant on nearby northern pin oaks. Frequent examinations were made throughout the spring, summer, and fall, but no fruiting was found on any of the hundreds of inoculated trees examined. Only 2 trees died during the 4 years. Since fruiting on northern pin oaks usually was observed only after the wood appeared moribund or dead, it may be that proper conditions for fruiting on bur oaks were not met. Ten bur oaks with extensive symptoms were cut in August and allowed to lie on the ground throughout the winter. No fruiting developed, even though the sapwood had darkened and the bark had loosened.

Samples of the infected sapwood of the above trees were taken periodically throughout the fall. Microtome sections (both transverse and radial) were examined for mycelial development such as that described in pin oak sapwood at the time of fruiting (20). No mycelial development was observed.

Discussion.—Various hypotheses concerning the mechanism of wilting in diseased plants were discussed by Beckman et al (3), with special reference to oak wilt. They proposed that plugging of the vessels by tyloses and gums and the resulting obstruction of water movement in the tree were primarily responsible for oak wilt symptoms. Other workers have demonstrated the presence of wilt-inducing "toxins" in culture filtrates of the oak wilt fungus (12, 13, 21).

Toxic substances would be expected to kill the host cells, particularly those cells adjacent to the vessels. As pointed out by Struckmeyer et al (19), however, the cells that gave rise to tyloses and the tyloses themselves were living cells. Such cells would be directly exposed to any toxic substances produced in the vessels. In bur oaks, the cells of the cambial region adjacent to the infected area remained alive and active. The infected roots of both pin and bur oaks showed no necrosis, and the roots of infected pin oaks continued to live for several years after the aerial parts of the tree had died (1, 22). The copious production of stump and trunk sprouts from infected bur oaks indicated that the adventitious buds were not injured. The above observations cast doubt on the presence of fungus "toxins" in the sapstream in quantities sufficient to damage the cells of woody and meristematic tissues; however, vascular plugging by tyloses and gums could account for the death of these tissues.

Water conduction to the affected parts of the trees was obstructed by vascular plugging in both pin and bur oaks. Comparison of disease development in the 2 species showed close similarities in the chain of events that led to foliage wilt; however, 2 major differences in disease development were observed. The entire crown of pin oaks wilted rapidly, and death of the aerial portions of the trees quickly followed. In bur oaks, only part of the crown was involved in most cases, and the trees generally lived for several years. The induction of foliage wilt by different degrees of trunk girdling helped clarify these responses.

In both northern pin oaks and bur oaks, ¾ of the trunk circumference could be girdled through the sapwood without visible effect on the leaves or on the cambium above the girdle. Thus, as little as ¼ of the circumference was able to supply water to the cambium of the other ¾, a situation analogous to the partial plugging of infected bur oaks. Complete girdling, analogous to entire vascular plugging in northern pin oaks, induced foliage wilt indistinguishable from that due to fungus infection, and the portion of the tree above the girdle was quickly killed. These observations suggested the following viewpoints: 1) the entire aerial portions of infected northern pin oaks died as a result of plugging extending through the entire circumference of the trunk; 2) infected bur oaks continued to live because a sufficient portion of the xylem remained open to supply water to the cambial region of the entire circumference; and 3) bur oaks died when the infection had spread through the tree to an extent that symptomatic plugging became analogous to a complete girdling of the sapwood. Thus, the distribution of vascular plugging in the tree appeared to determine disease severity.

The close correlations among fungus distribution, vascular plugging, and foliage wilt indicated that the fungus induced disease reactions only in invaded tissues. Differences in movement of dyes in the respective vascular systems and the spread of the fungus from localized inoculations suggested that the fungus spread tangentially through the xylem of northern pin oaks much more rapidly than through the xylem of most bur oaks. Thus, factors that modified tangen-

tial movement of the fungus within the host appeared to govern the extent of vascular plugging and, hence, the differences in disease severity between the 2 species.

Recovery from infection by the oak wilt fungus has not been reported in the red oak group. Recovery has been reported, however, in members of the white oak group (21). Of the bur oaks in which year-to-year progress of the disease was followed in the present study, 46 per cent failed to develop symptoms during the second or succeeding years. The ability of the host to form a healthy new annual ring over the infected ring contributed to this recovery.

A new annual ring formed in the year following initial infection in all bur oaks examined except 2 trees that died the first year. Following the formation of the new ring, the fungus was, in effect, buried in the wood. Before wilt developed again in the second year, the fungus had to invade the new annual ring and re-establish itself in the actively conducting vessels. No evidence of beetle injury was found in bur oaks that recovered from the disease; whereas, beetle galleries frequently were found in trees with recurring symptoms. Thus, it appeared that beetle galleries or other injuries to the vascular system might provide a pathway by which the fungus could invade the newly formed annual ring. In the absence of such injuries, the fungus would remain confined to the buried ring and no further symptoms would develop. Isolations made from recovered bur oaks indicated that the fungus died out within 1 to 2 years in such "buried" infections. Thus, trees that failed to show recurring symptoms appeared to offer little menace to the surrounding trees.

Several inferences regarding the occurrence and spread of oak wilt in bur oaks have been drawn from the available data. Studies in Iowa (4, 21) and Wisconsin have indicated that mortality among bur oaks is much less than among pin or red oaks. Three factors appeared to contribute to this lower mortality: 1) The difficulty in securing infection of bur oaks by standard inoculation techniques suggested that bur oaks may often escape natural infection. 2) Because of the infrequency of root-grafting among bur oaks, trees adjacent to infected bur oaks seldom became infected; in contrast, many pin and red oaks frequently died as the result of infection of 1 tree. 3) Many bur oaks recovered from infection, whereas recovery did not occur among pin and red oaks. Thus oak wilt in bur oaks appeared much less damaging to forest stands than oak wilt in pin or red oaks. These inferences may have a bearing on control plans.

A rapid method of determining oak wilt without the necessity of making isolations would be valuable. The presence of tyloses in the vessels of the outer annual ring of oaks infected with the oak wilt fungus is easily determined. This constant association of tyloses with the disease is a valuable aid in the identification of oak wilt in the field.

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IDENTITY OF THE OAT RED-LEAF VIRUS WITH THE BARLEY YELLOW-DWARF VIRUS [1]

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SUMMARY

A possible relation between the barley yellow-dwarf virus and the oat red-leaf virus has been suspected since 1952.

Experiments were conducted with red-leaf-affected oat plants obtained from 33 different locations in Illinois and from 1–2 locations in each of the following states: Georgia, Iowa, Maine, Massachusetts, Minnesota, New York, South Dakota, Washington, and Wisconsin. Experiments also were carried out with yellow-dwarf-affected barley plants obtained from California, Illinois, Iowa, Minnesota, South Dakota, Wisconsin, and Washington. Identity of the red-leaf virus with the barley-yellow virus is proposed on the basis of the similarity of the 2 viruses in regard to symptomatology, incubation period in the host plant, aphid transmission, persistence of the virus in the aphid vector, and failure to transmit either virus through seed or soil or by mechanical means.

Red leaf, a virus disease of oats, has caused considerable loss in yield in many of the oat-growing sections of the United States. The disease is characterized by a reddening of the leaves, an over-all stunting of the infected plant, and a blasting of the florets. Leaf reddening in oats is not peculiar to this disease alone. Sprague (19, 20, 21) and McKinney (7, 8) have emphasized that reddening may be caused by such diverse factors as low temperature, poor soil drainage, aphid feeding injury, low nitrogen and phosphorus supply, and various fungi.

Although red leaf undoubtedly has been present for a long time (4, 5, 6, 17, 18), its etiology has been demonstrated only recently. Oswald and Houston (10, 11, 12, 13, 14, 15) first reported the existence of a cereal disease caused by a virus that was readily transmitted by aphids to oats, barley, wheat, and rye and that was neither soil borne, seed borne, nor mechanically transmitted. They named the disease yellow dwarf, since yellowing and dwarfing were the outstanding symptoms on barley and wheat. The main symptoms on oats were a leaf-reddening, a stunting of the entire plant, and a blasting of the florets—symptoms typical of the so-called red-leaf disease.

Subsequently, a number of investigators (9, 22, 23) have reported transmitting a virus from red-leaf-affected oat plants by means of aphids but failure to do so by mechanical means. The identity of the red-leaf virus and the yellow-dwarf virus was not determined by these workers. Moore (9), working with red-leaf-affected oat plants, reported the successful transmission of the virus to oats by apple-grain aphids and by grain aphids. Wilson and Murphy (24), also working with red-leaf-affected oats, obtained successful transmission of the virus by the apple-grain aphid to oats but not to barley. They reported that the virus was transmitted through soil but not through seed or by mechanical means. Summers (22) found that the red-leaf virus was readily transmitted by the greenbug and by the grain aphid to oats but not to Hardired wheat. Although the published results of the various

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investigators differ in regard to host range and transmission through soil, a possible relationship of the viruses to each other is clearly suggested. All of the viruses were readily aphid transmissible and produced similar symptoms in oats—leaf-reddening, stunting, and floret blasting.

The purpose of this investigation was to determine the relationship of the red-leaf virus of oats to the yellow-dwarf virus of cereals. An abstract of this paper has been published (23).

METHODS AND MATERIALS.—Studies were carried out in 3 greenhouse sections that were sprayed frequently with Malathion to control insects. Greenhouse temperatures could not be controlled from April through October, but during November through March the temperature in 2 of the sections varied from 62° to 75°F. During the fall and winter, supplemental illumination of approximately 600 foot-candles was provided daily from 6 a.m. to 8 p.m. Test plants were grown in a pasteurized soil-sand-manure mixture in 4-, 5-, or 6-in. clay pots, 2–4 plants per pot.

Mechanical inoculation usually was made by the Carborundum-abrasion technique. The plants were washed with tap water immediately after inoculation with sap extracted from several leaves that showed incipient or moderately advanced disease symptoms. Inoculated plants were kept for 8–10 weeks.

In the aphid transmission studies, 2 types of cages were used. One type was made by stapling together 2 edges of a sheet of clear cellulose nitrate to form a cylinder. To provide ventilation and to make the cage aphid-proof, 1 end of the open cylinder and 2 windows on the sides were covered with fine Lumite plastic screen. The cage was placed over plants growing in pots and held in place by inserting a portion of the open end of the cage into the soil. Such cages were used to confine aphids on plants during acquisition and inoculation feeding periods, to raise and confine nonviruliferous aphids on healthy plants, and to cover newly emerged seedlings. Small individual-leaf cages, made of balsa, cork, and Lumite screen according to the method of Eide and McLean (3), were used primarily in tests for the presence or absence of virus. About 5–10 nonviruliferous aphids were placed in each leaf cage, and the aphids were allowed to feed directly on a leaf of a test plant for at least 18 hours. The aphids were then transferred to healthy seedlings and allowed to feed for 1–3 days before being killed with an insecticidal spray.

Virus-free aphid colonies were established as follows: 10–20 winged aphids of a single species were placed in a petri plate containing a healthy small-grain seedling, the seedling was examined every 1–2 hours, and any newly emerged nymphs were transferred by means of a camel's-hair brush to healthy oat plants. Colonies started in this manner have always proved to be free of virus and have been maintained on caged plants of either winter oats or barley. The aphids were transferred about every 3–5 weeks to healthy plants and the colonies tested for the presence or absence of virus. Mass transfers of aphids were made by dislodging aphids from plants onto a sheet of aluminum foil that was folded once, then held above the plant to be infested and tapped lightly. Transfer of single aphids was made by means of a moistened camel's-hair brush.

Transmission tests with the various species of aphids were conducted in the following manner. Virus-free aphids of a particular species were placed upon diseased plants and allowed to feed for varying periods (acquisition feeding period), usually 3 days. The aphids were then removed and placed upon healthy small-grain seedlings, usually 3–5 aphids per plant, and allowed to feed for an additional period of 3 days (inoculation feeding period). A parallel series was conducted at the same time with virus-free aphids that were fed on healthy instead of diseased plants during the first feeding. All aphids in any test were confined to seedlings during their acquisition and inoculation feeding periods by means of the cylindrical cellulose nitrate cages previously described. Following the desired inoculation feeding period, the cages were removed and the aphids killed by means of a dilute Malathion spray. Stock cultures of the virus were maintained in plants of Dubois oats, Tennessee Winter barley, and Blackhulless barley, each grown in insect-proof cages.

Most of the following small-grain varieties and selections whose reactions to the yellow-dwarf virus are described by Oswald and Houston (13) were used throughout these trials as indicator plants: 1) barley—Blackhulless (C. I. 666), C. I. 734, and C. I. 998; 2) oats—California Red (C. I. 1026), Coast Black (C. I. 1025), and Traveler (C. I. 4206); 3) wheat—Baart 46; and 4) rye—Merced. The oat varieties Dubois (C. I. 6752) and Sante Fe (C. I. 4518) as well as the wheat variety Lee (C. I. 12488) also were used in some of the trials.

TRANSMISSION EXPERIMENTS WITH APHIDS COLLECTED FROM THE GREENHOUSE AND THE FIELD.—Red-leaf-affected oat plants growing in the greenhouse were first pointed out to the writer by W. M. Bever in March, 1954. The plants were heavily infested with apple-grain and grain aphids (*Rhopalosiphum prunifoliae* and *Macrosiphum granarium*, respectively), both species of which have been reported to be vectors of the yellow-dwarf (2, 10, 11, 14) and the red-leaf (9, 22, 23) viruses. The 2 species of aphids were collected separately and placed upon healthy oat seedlings in individual-plant cages (3–5 aphids per plant). The aphids were killed with an aphicidal spray 5 days after infestation. At the end of 23 days, 12 of 20 plants infested with *R. prunifoliae* and 16 of 20 plants infested with *M. granarium* developed red-leaf symptoms. None of the 20 noninfested control plants became diseased. No infection resulted from mechanical inoculation with sap from ground oat leaves. Barley, wheat, and rye, as well as oats, were included in some of the subsequent transmission studies. A total of 7 aphid collections were made in the field from red-leaf-affected oat plants growing in widely

separated areas of Illinois. The aphids were placed, 3–5 aphids per plant, on healthy small grain seedlings for an inoculation feeding period of 3 days. The virus was transmitted to oats, wheat, barley, and rye with 3 collections but not with the other 4 collections. The incubation period varied from 2 to 3 weeks. Red-leaf symptoms developed on oats that, except for a slightly reduced reddening, were typical of the disease in the field. Barley and wheat plants developed a slight-to-moderate yellowing of the leaves and a moderate stunting of the plants. Rye, except for a slight stunting, appeared normal. Recovery tests (starting with virus-free aphids) to appropriate indicator plants confirmed the presence of the virus in barley, wheat, and rye, as well as in oats. The symptoms on oats and rye were identical to those described by Oswald and Houston (14) for yellow-dwarf disease of oats and rye. The symptoms on barley and wheat were of the same type as those described by Oswald and Houston (14) for the same barley (Blackhulless) and wheat (Baart 46) varieties, but they were less severe.

TRANSMISSION EXPERIMENTS WITH APHIDS REARED VIRUS FREE.—Virus-free colonies, originally collected in the field from various small grains and grasses, were eventually established of the following species: apple-grain aphid, *Rhopalosiphum prunifoliae* (Fitch); grain aphid, *Macrosiphum granarium* (Kirby); green-bug, *Toxoptera graminum;* and the corn aphid, *M. maidis* (Fitch).

The results of transmission trials involving the 4 aphid species and many collections of diseased plants demonstrated that a virus was recovered from red-leaf-affected oat plants collected from each of 33 widely separated fields in Illinois, and from 1 or 2 collections obtained from each of the following states: Georgia, Minnesota, Wisconsin, Iowa, South Dakota, New York, Maine, and Massachusetts. All of the 44 isolates produced typical red-leaf symptoms in oats. These symptoms were identical to field symptoms and to those described by Oswald and Houston (14) for yellow-dwarf of oats. All 44 isolates caused typical yellow-dwarf symptoms on barley, wheat, and rye, as well as on oats. As this work progressed, some variability in virulence among the different isolates of the virus became evident. For example, some isolates on barley and on wheat caused only a slight to moderate stunting and a slight yellowing of the leaves, whereas a highly virulent isolate caused a very severe stunting of the plant and very severe leaf yellowing.

Efficiency of transmission varied from a low of 16 per cent to a high of 100 per cent, depending upon the species of aphid and on the conditions used. No comparison can be made of the transmission efficiencies of the different species, since the acquisition and inoculation feeding times as well as the condition of the source plant varied with the different trials.

Field collections of barley and wheat plants manifesting yellow-dwarf symptoms were obtained from single locations in Wisconsin, Minnesota, South Dakota, and Iowa and from 3 locations in Illinois. Almost invariably, red-leaf-affected oat plants were found adjacent to such plantings. All of the virus isolates from Illinois, Iowa, Minnesota, South Dakota, and Wisconsin have caused typical yellow-dwarf symptoms in oats, wheat, barley, and rye. A comparison of the oat red-leaf virus with known isolates of the yellow-dwarf virus from California and Washington was considered after 15 months' work—at which time it appeared fairly certain that the 2 viruses are identical. This precaution was considered necessary at that time since the presence of the barley yellow-dwarf virus had not been established in states other than California and Washington. Except for the fact that the California and Washington yellow-dwarf isolates appeared to show quantitative differences in virulence when compared on the same small-grain varieties, they were indistinguishable from the red-leaf isolate from oats.

Attempted mechanical transmission. — Mechanical transmission studies were conducted with red-leaf-affected oat plants obtained from 10 locations in Illinois; with single collections from Iowa, Wisconsin, and Minnesota; and with diseased barley and wheat plants from 3 locations in Illinois. Additional mechanical inoculation experiments were conducted with diseased barley, wheat, oat, and rye plants that had been infested a month earlier with aphids fed previously on a red-leaf-affected oat plant. The diseased source plants were tested by means of aphids for the presence of the virus at the time of inoculation. The Carborundum-abrasion technique was used in all mechanical transmission trials. In some experiments, the method of Yarwood (25) also was used. In such tests, several strips of diseased tissue were held together by forceps, and the cut edges of the diseased leaves were rubbed lightly several times over the surface of healthy leaves. Six to 12 seedlings of each variety of test plant were used in each test. None of the inoculated oat and barley seedlings became infected in these trials; in addition, rye and wheat were used as test plants in 6 of the trials, and none of these became infected.

In further inoculation trials, the usual procedure in the Carborundum-abrasion technique was modified in the following ways: 1) preparation of inoculum, inoculation, and incubation (for 2 days) at 41°F, with subsequent incubation for 3 weeks at 65°F; 2) preparation of inoculum and inoculation at a temperature of 65°F, followed by incubation at constant temperatures of 65°, 75°, and 82°F; 3) dilution of inoculum consisting of ground diseased leaves from one of 1:10 to a maximum of 1:100,000; and 4) the addition of 0.1 M or 0.01 M sodium sulfite, of 0.5 M, 0.1 M, or 0.01 M sodium chloride, or of a neutral phosphate buffer made up of KH_2PO_4 and K_2HPO_4. In each case, inoculum was prepared from leaves of either barley, wheat, or oats. Six to 12 seedlings of wheat, of rye, of oats, and of barley, each in the 2–3 leaf stage, were inoculated in each test. Positive results were not obtained in any of the experiments. Other observations

indicate that the virus is not readily transmitted mechanically. In several experiments, red-leaf-affected oat plants and healthy oat plants were grown in the same pot. The healthy plants have always remained disease free, even though in many instances leaves with red-leaf symptoms have been placed directly on the the leaves of healthy oats and left there for long periods. The same has been found to be true in tests with barley and wheat plants. No successful transmission resulted when air currents from electric fans were directed at diseased and healthy plants in a manner that caused the leaves to rub together.

TESTS FOR POSSIBLE SEED TRANSMISSION.—In order to determine whether or not the virus could be transmitted through the seed, oat seeds were collected and tested from the following plants 1) field-grown plants thought to be affected with red leaf (1463 seeds); 2) greenhouse-grown plants known to be affected with red leaf (609 seeds); and 3) greenhouse-grown plants, the panicles of which when first headed had been heavily infested with viruliferous grain aphids (455 seeds). The seeds were sown in pasteurized soil in clay pots or in wooden flats. Whenever possible, seeds of the same variety collected from virus-free plants were used as controls. Of a total of 2527 seeds sown, 2364 seeds germinated (93.5 per cent). At the end of 8–10 weeks, none of the oat plants had developed red leaf.

In addition, none of the 245 barley seeds and 173 wheat seeds taken from diseased plants previously infested with aphids that had fed on red-leaf-affected oat plants developed symptoms when planted. The results indicate that the virus is not seed borne in oats, barley, or wheat.

TESTS FOR TRANSMISSION THROUGH SOIL. — Soil samples of many types were collected in various parts of Illinois from 22 oat fields, containing plants showing red leaf. The samples were taken from soil immediately surrounding oat plants affected with red leaf. The soil was placed in wooden flats, 20 × 15 in., and stored in a cold storage room until needed. The usual procedure was to plant 25 virus-free oat seeds per row, 10 rows per bat, alternating 5 rows of Clinton 11 and 5 rows of Santa Fe. The soil in each flat was fertilized with either a 4-10-10 or 10-10-10 fertilizer mixture, depending upon the need. The plants were kept until the heading stage. No evidence was obtained that the virus is transmitted through the soil; however, plants growing in certain soils obtained from southern Illinois manifested anthocyanin disturbances resulting from nitrogen and phosphorus deficiencies. Certain of these plants were tested by means of virus-free aphids, but a virus was not recovered.

Additional tests were conducted with soil in which red-leaf-affected oat plants had been grown to maturity in the greenhouse. The tops of the plants were removed, the roots shredded, and the latter worked thoroughly (and as evenly as possible) into the soil. The soil was then placed into 54 earthenware crocks, watered, and allowed to stand a month before being planted to Santa Fe oats (5 seed per pot). Twelve of the pots had been pasteurized for 40 minutes at 15 lb. pressure previous to seeding and were used as controls. At heading, none of the plants had developed red leaf, and the experiment was terminated. These results indicate that the virus is not transmitted through the soil.

PERSISTENCE OF THE VIRUS IN M. GRANARIUM AND R. PRUNIFOLIAE.—Oswald and Houston (14) reported that yellow-dwarf virus persists in aphid vectors for at least 5 days. Consequently tests of persistence in vectors were made with the red-leaf virus.

Nonviruliferous winged apple-grain and grain aphids were placed on red-leaf-affected oat plants and allowed to feed for 3 days. At the end of this time each species was transferred, at the rate of 10 aphids per plant, to 5 healthy oat seedlings in the 2–3 leaf stage. Following a 24-hour inoculation feeding period, the aphids were transferred to a second series of 5 healthy plants. This procedure was repeated at 24-hour intervals for 15 days. All of the plants on which aphids were placed became infected, an indication that the virus persisted in both *M. granarium* and *R. prunifoliae* for 15 days, the maximum time tested.

DISCUSSION.—That the red-leaf virus of oats and the yellow-dwarf virus of cereals are identical is proposed on the basis of the following points of similarity: 1) identical symptoms produced by the 2 viruses on oats, barley, wheat, and rye; 2) identical incubation periods of the 2 viruses; 3) transmission of both viruses by means of the same aphid species (*M. granarium*, *T. graminum*, *Rhopalosiphum prunifoliae*, and *M. maidis*); 4) similar persistence of the 2 viruses in *M. granarium* and *R. prunifoliae*; 5) failure to transmit either virus by mechanical means; 6) failure to obtain transmission of either virus through the soil; and 7) failure to obtain seed transmission of either virus. Therefore, it appears highly desirable to obtain further evidence from serological, cross-protection, and virus characterization studies.

Variation in virulence among the different isolates of the virus became apparent as this work progressed. No attempt was made to determine whether or not a virus isolate was composed of a single strain or a mixture of strains. It was obvious, however, that the difference in virulence among the various isolates was of sufficient magnitude to justify a warning that precaution be exercised against the indiscriminate use of yellow-dwarf strains in a varietal evaluation program.

Red leaf, as it has been pointed out, is sometimes difficult to distinguish from leaf reddening caused by other factors. When red-leaf-affected oat plants are grown under favorable temperatures (55–75°F) and under high light intensities, red leaf usually can be distinguished from ordinary leaf reddening. It is believed that the most important distinguishing symptom of red leaf is the appearance of light green blotches, which are characteristically the first symptoms of the disease. Under conditions of high temper-

ature and low moisture, however, the areas covered by the blotches may die rapidly; under such conditions, transmission tests with aphids are the only reliable diagnosis.

That the yellow-dwarf virus occurs in Georgia, Iowa, South Dakota, Maine, Massachusetts, and New York is reported for the first time. Its presence in California, Illinois, Minnesota, Wisconsin, and Washington was confirmed. This is additional evidence in support of the recent reports by Allen and Houston (1) and of Oswald and Thung (16) of the widespread occurrence of the virus.

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THE MORPHOLOGY AND LIFE HISTORY OF PHYLLACHORA PUNCTUM [1]

C. R. Orton [2]

SUMMARY

The first known report of a successful artificial inoculation of a host by ascospores of a species of *Phyllachora* is presented here for *P. punctum* on *Panicum clandestinum*. Spermagonia developed 16–24 days after inoculation, and mature perithecia bearing ascospores were present 6–10 days later.

Perennial species of *Panicum* observed in early spring showed small infections on living basal leaves, indicating that *Phyllachora punctum* passes the winter in this stage. These infections produced ascospores readily when plants were brought into the greenhouse.

The immature ascus of *P. punctum* has a double wall, the outer wall forming a thickened apical cap that is blown off with ascospore expulsion. Only a part of the ascospores are discharged in this expulsion. The asci, with the remaining ascospores, are then forced out of the ostiole and collect on the surface as sticky droplets.

In water and on agar media the ascospores germinate, form a germ tube terminated by an appressorium-like swelling, and cease to develop.

The genus *Phyllachora* was established by Fückel (3) and was placed in the family Dothidiaceae, characterized by the formation of ascocarps in a carbonaceous stroma embedded in the mesophyll of the host leaves and lacking true perithecial walls.

Orton (8) demonstrated that *Phyllachora graminis* (Pers.) Fckl., the type species of the genus, is not stromatic. The stromatic appearance is caused by the crowding together of the perithecia in the compound fructification, resulting in the walls being frequently in contact. Furthermore, the formation of the clypeus as an outgrowth of hyphae from the upper part of the perithecial wall gives the appearance of a true stroma when fully formed. Each perithecium possesses a true perithecial wall, and the genus, as typified by *P. graminis*, is not dothideoid but is allied with the Sphaeriales. These facts have been verified independently by Petrak (10) and by Miller (5). Later, Miller (7) showed that *P. ambrosiae* (Berk. & Curt.) Sacc. is very similar in structure to *P. graminis* in producing a clypeus composed of dead epidermal cells and hyphae of the fungus, with no true stroma. *P. lespedezae* (Schw.) Sacc. differs in having perithecia embedded in a solid fungus stroma (6).

Authors of modern textbooks of mycology do not generally agree as to the position of the genus *Phyllachora* in the system of classification. Wolf and Wolf (11) recognize a family Phyllachoraceae but retain it in the order Dothidiales, coordinate with the family Dothidiaceae. Gäumann (4) places *Phyllachora* together with *Polystigma* in the family Polystigmataceae of the order Sphaeriales, stating that "the perithecia of these genera are embedded in a fleshy or cartilaginous stroma." Both Bessey (2) and Alexopoulos (1) place the family Phyllachoraceae at the end of the Sphaeriales and interpret the clypeus as a stroma.

Miller (6) describes the perithecia of the Phyllachoraceae as being "globose, with thin walls, lying in leaf tissue or in a stroma and maturing in living leaves, with plane ostioles and sometimes with a clypeus formed by the lateral proliferation of ostiolar hyphae."

The writer believes that the nearest affiliate of the Phyllachoraceae is to be found in the Clypeosphaeriaceae of the Sphaeriales.

The writer (9), in a taxonomic treatment of Phyllachora on grasses in North America, reported what appeared to be spermatia and conidia associated with the ascigerous stage of several species. No proof of the connection of either of these 2 stages with the perithecia exists up to the present time.

In May, 1953, collections of overwintered plants of *Panicum clandestinum* L. bearing *Phyllachora punctum* (Schw.) Orton of the previous season's infection revealed on examination only the dissipated remains of the compound fructification of this fungus. Several host plants showing new growth were dug and brought into the greenhouse where they were potted. On further examination of these plants, it was discovered that the fungus was fruiting on the basal leaves that had overwintered in the living condition protected by old leaves and plant debris (Fig. 1). When the fruit bodies on these leaves were examined microscopically, it was found that the perithecia were filled with mature asci and ascospores. In fact, they were exuding both asci and ascospores through the ostioles; the asci and ascospores collected in whitish masses clearly visible on the surfaces of clypei (Fig. 2).

The same condition of the fungus was found on plants of *Panicum huachucae* Ashe and of *P. sphaerocarpon* Ell. brought into the greenhouse from other locations. So it is evident that *Phyllachora punctum* overwinters by infecting in the fall the basal leaves that are formed on these perennial hosts. These infections carry over during the winter and are the source of the general infections that develop during the summer. When the summer- and fall-infected

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[2] This paper was prepared after the death of the author from his notes, drawings, and partially completed manuscript. It was completed by H. L. Barnett and is presented without discussion.

leaves die the fungus on them also dies.

When freshly produced ascospores were sown on plants of *Panicum clandestinum* and *P. sphaerocarpon* that were kept under belljars lined with moist absorbent paper, infected areas were visible after 16–24 days. The first signs proved to be spermagonia that developed as flask-shaped bodies under the epidermis, with quite noticeable clypeal formation in the epidermis and immediately beneath it (Fig. 3). The spermagonia produced copious spermatia, measuring 0.5 × 7.0–10.0 μ, which exuded through an ostiole in delicate cirrhi. Attempts to germinate the spermatia and to establish infection in the host by using them as inoculum failed. Perithecia containing mature asci developed in 6–10 days following the appearance of spermagonia. Mature perithecia of an old infection are shown in Figure 4. In some cases the development of spermagonia continued quite extensively within the mesophyll tissue without ascocarp formation (Fig. 5). This phenomenon suggests a possible heterothallic condition in *Phyllachora punctum*. No conidial stage has appeared in these cultures, and it seems probable that the conidia found in the fructifications of this species (9) belong to a fungus that invades the old perithecia.

At maturity the asci are forcibly discharged from their base, apparently by the pressure of the successively developing younger asci. Observations of mature perithecia showed asci pushing through the ostiole, some with 2, 4, or 6 spores discharged and the remaining spores still retained in the asci. When a Petri dish containing agar was inverted over a ma-

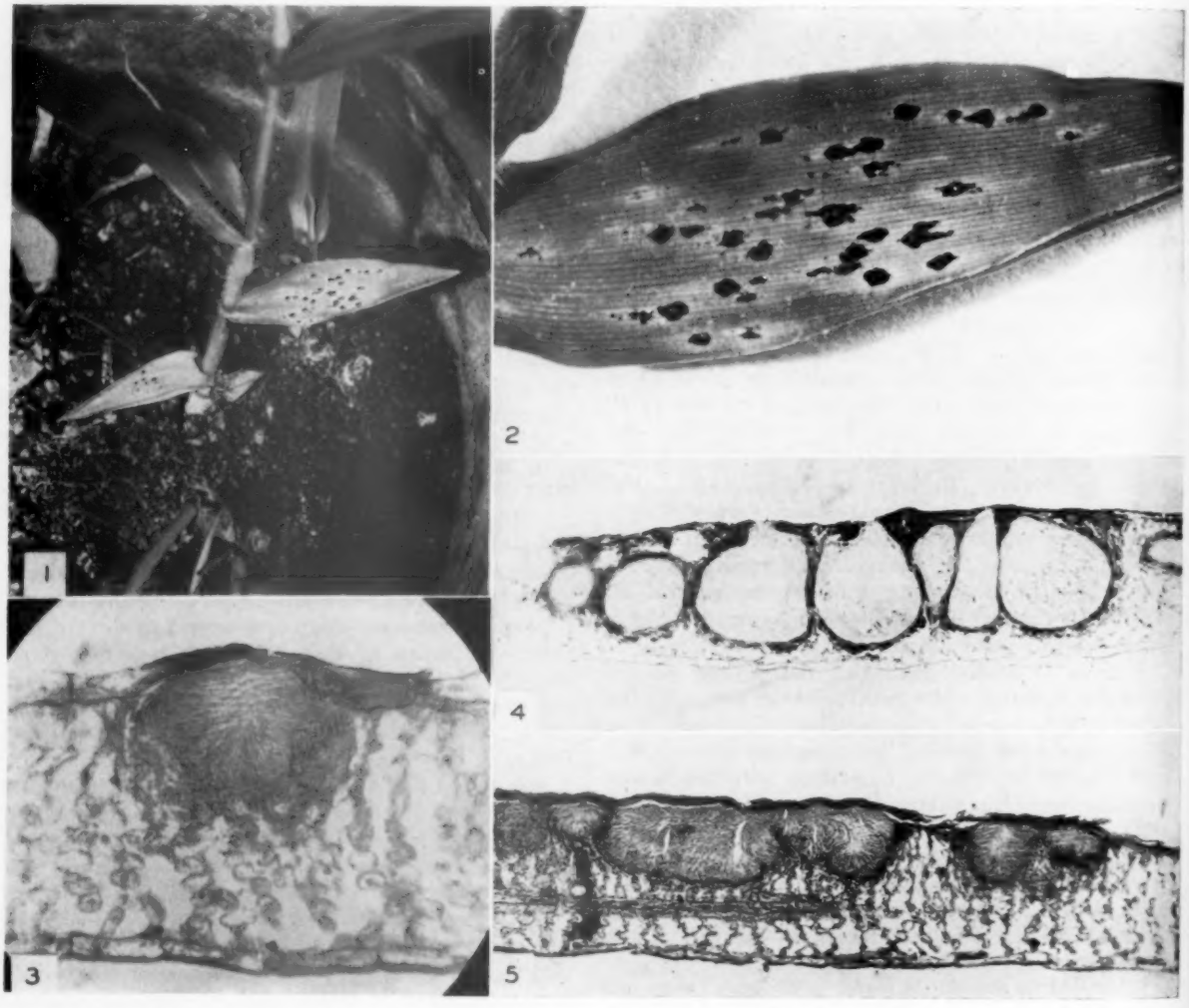

FIG. 1–5. *Phyllachora punctum* on *Panicum clandestinum*.—Fig. 1. Infected areas on lower 3 leaves that had lived over winter in nature. Approximately ×½.—Fig. 2. Enlargement of leaf in Figure 1, showing extruded asci and ascospores. Approximately ×2.—Fig. 3. Section of leaf and spermagonium showing early stage in the formation of clypeus in epidermal cells. Approximately ×200.—Fig. 4. Section of leaf showing mature perithecia and extensive clypeal development. Approximately ×80.—Fig. 5. Section of leaf showing extensive development of spermagonia without formation of perithecia. Approximately ×100.

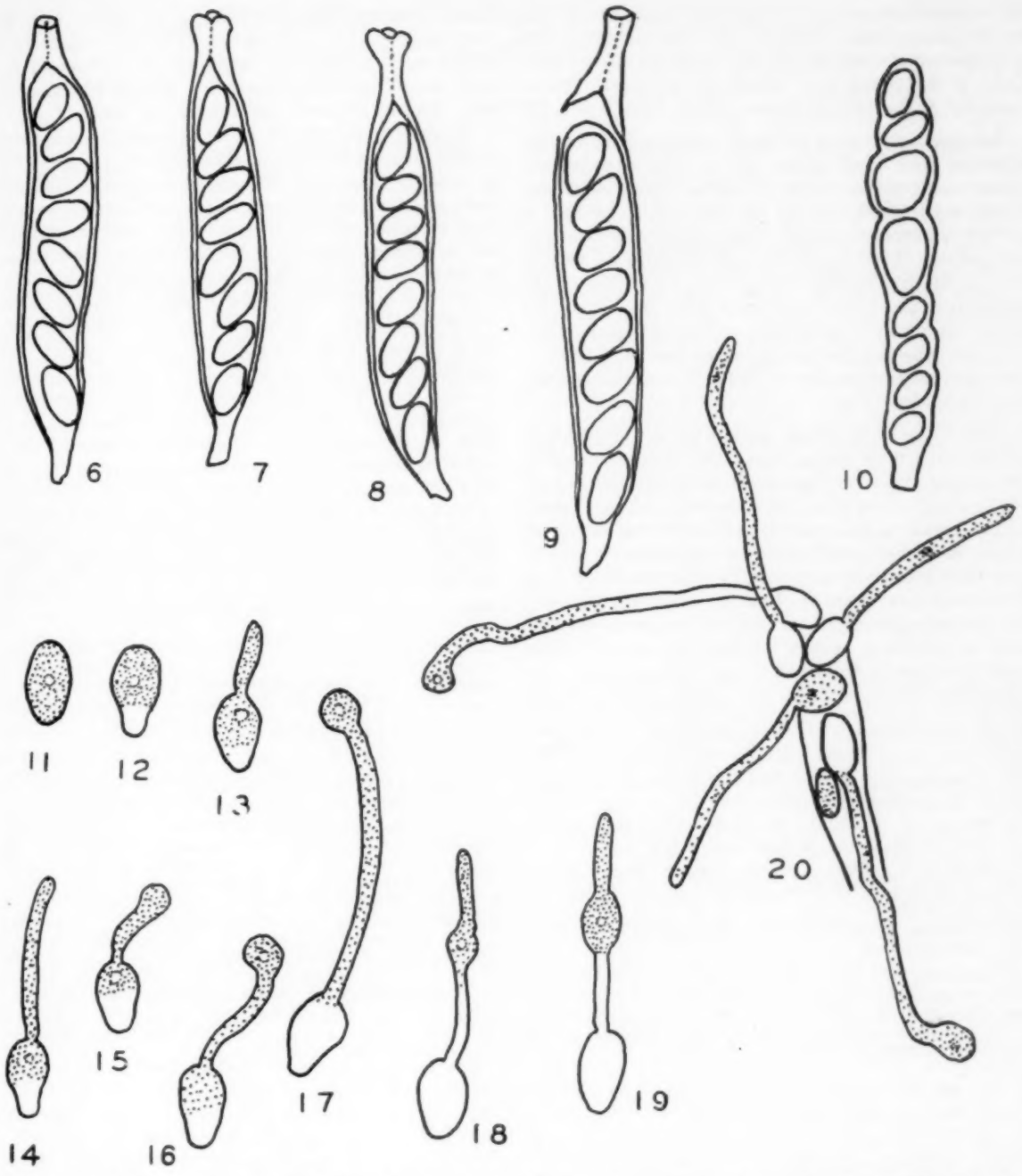

FIG. 6–20. Drawings showing morphology of asci and germination of ascospores. Approximately ×1200.—Fig. 6–8. Immature asci showing double wall and thickened apex.—Fig. 9. Mature ascus showing expulsion of thick apical cap.—Fig. 10. Mature ascus with 2 swollen ascospores prior to germination.—Fig. 11–12. Swollen ascospores in process of germination.—Fig. 13–15. Germinated ascospores.—Fig. 16–17. Appressorium-like structures at apex of germ tubes.—Fig. 18–19. Short secondary germ tubes from the enlarged apical bodies.—Fig. 20. Germination of ascospores within ascus and growth of germ tubes through the ascus wall.

ture perithecium in the process of discharging ascospores, it was found that about 75 per cent of the discharged ascospores were in pairs, a lesser number in groups of 4 or 6, but no case was observed where all 8 ascospores were discharged together. The sticky substance covering the ascospores held them together in clumps as they were discharged, and only an occasional single spore was observed.

Few studies of the morphology of the ascus in *Phyllachora* have been made (6, 7) but it is evident that

the structure of the ascus is not uniform in all species. In the graminicolous species, with few exceptions, no well-developed terminal pore is evident in mature asci. Only in the young asci, before the ascospores reach maturity, is the terminal pore evident (Fig. 6–8).

An examination with the phase microscope of freshly extruded asci of *P. punctum* in water showed the young ascus to possess a double wall, the outer one being much thickened at the apex (Fig. 6–9). A narrow channel extends to a pore in the center of the concave top of the thickened apex. It seems anomalous that in the developing ascus a pore is visible in the specially formed thickened apex, through which one would naturally expect the ascospores to be discharged, whereas the mature ascus has no thickened apex and no pore is present in the thin wall surrounding the spores.

The failure of the asci to show evidences of explosive power after their escape from the perithecium indicates some explosive function initiated within the perithecium. The initiation, development, and maturation of the asci is a progressive and continuous operation under favorable conditions. As their spores mature, the older asci must give way to this succession, and they break away from the base of the perithecium and are pushed upward to the ostiole. It seems possible that as the ascus pushes through the narrow ostiole some pressure is developed at its tip, resulting in the expulsion of the thickened cap and the release of the upper spores. Then the less turgid ascus, with its remaining spores, is extruded. The asci collect in a sticky mass outside the ostiole on the surface of the leaf, where the remaining spores may be disseminated with the aid of water and possibly by insects.

Initial germination of ascospores taken from fresh perithecia was secured in about 2 hours at 25°–27°C in distilled water and reached a maximum of 80 per cent in 24 hours. Several agar media served equally well as substrates for germination. The first evidence of germination was a swelling of the ascospores from a normal size of 4.5–5.5 × 9.0–12 μ to 7.0–8.7 × 14–17.5 μ (Fig. 10–12). It was noted that the spores on swelling frequently became pyriform, and the germ tube emerged from the larger end (Fig. 13–15). Occasionally the ascospores appeared constricted at the middle as though 2-celled, but no septum developed. The germ tube sometimes reached a length 5 times that of the spore and frequently terminated in a thick-walled ovate body like an appressorium (Fig. 16, 17). No further development occurred on agar media or in water, except that an occasional short secondary tube arose from the terminal body (Fig. 18, 19). Germination of the ascospores occurred readily within the mature ascus (Fig. 10, 20). In such cases the germ tube passed through the ascus wall.

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HOST NUTRITION AND ATTACK BY FUNGAL PARASITES[1]

John Grainger

SUMMARY

The term "disease potential" has been used to describe the relative physiological ability of a host crop or plant to contract disease. Oat plants have 2 well-known periods of high disease potential to Helminthosporium leaf spot (the primary and secondary phases of disease) with a period of low potential between. The potato has been shown to behave in a similar manner to Phytophthora blight, though the first period of high potential is not usually seen in the field in Britain. Carbohydrate, nitrogen, and ash were estimated in different plant organs of both hosts throughout the growth cycles of each in order to see if changes in host nutrition or composition would account for such variation in behaviour towards the fungal parasite.

Periods of low disease potential in both hosts were found to be the times of most rapid growth and also of low percentage of total carbohydrate in the whole plant, and *vice versa*. Joint effects of these 2 factors were assessed by calculating the "C_P/R_S ratio"—the weight of total carbohydrate in the whole plant (C_P) divided by the residual dry weight of the shoot (R_S). High ratios were correlated with high disease potentials and *vice versa*. This relation was further tested by growing oats and potatoes at different times of the year so that plants having widely different ratio values grew near to one another. When disease occurred, it developed rapidly and severely on high-ratio hosts, whereas crops having low ratios either were healthy or were attacked only in minor degree.

The behaviour of either host to disease was, indeed, always according to its ratio value. "Zero time" in blight forecasting denoted the time when ratios began to rise and the crop to become increasingly able to contract epidemic disease. Subsequent rates of blight development were higher, the higher the ratio values involved, and *vice versa*. Intermediate ratio values of "dormant" tubers indicated their intermediate disease potential in relation to that of the growing plant. Ratio values were found to be unrelated to inherent varietal susceptibility.

Certain host-parasite relations are known where disease occurs regularly at particular stages of the host's growth-cycle and not at others. Primary and secondary phases of leaf spot (caused by *Helminthosporium avenae* Eidam) of oats are separated by a period of relatively slight disease, or even of healthy growth. When forecasting the first appearance of potato blight (caused by *Phytophthora infestans* (Mont.) d By.) by climatic criteria, it was found necessary to specify a "zero time" before which the disease did not occur. Such occurrences of healthy growth at one stage and disease susceptibility at another merit investigation, and the present work was accordingly designed to see if the composition of the host varied with time in any manner that could be linked with the differing susceptibility to disease.

The writer, working with Beaumont's specification for blight forecasting (2), was able to forecast the first appearance of blight very well, provided that "Beaumont periods" occurring in June were not regarded as valid (7, 8). This led to the suggestion of July 1 as a "zero time" for the validity of blight forecasts in west Scotland, and Large (12) has found a similar necessity in England and Wales (the last week in June). The apparent insusceptibility of the crop to blight in June was not invariably due to lack of infection, for small lesions have been found on the foliage as early as May 27 (9). Such early "token" infections do not, however, develop until later, even if a period of suitable climate occurs in June. Although Beaumont's specification gave accurate forecasts of the first appearance of blight, there was no consistent relation between the occurrence of subsequent "Beaumont" periods and the rate of development of blight epidemics (7, 8).

PREVIOUS WORK ON "DISEASE POTENTIAL". — The terms "susceptible" and "resistant" denote the basic ability of a plant, variety, or clone to become diseased. Some other term is necessary to describe such physiological reactions to disease as those that vary according to the age or state of development of a host. Beaumont (1) reported the results of a large volume of work on potato that he interpreted as not proving any change of "inherent susceptibility" to Phytophthora blight with age of the host. There were, nevertheless, marked variations in what he called "epidemic potentiality" of the crop, which, however, he thought due to external factors. A new concept—"disease potential"—suggested by Beaumont's "epidemic potentiality" but apparently quite different from it—has been adopted in the present paper to denote the relative physiological ability of a host crop or plant to contract disease at different stages of its life history.

Leaf spot of the oat regularly occurs in well-recognized primary and secondary phases separated by a period of healthy growth or of relatively slight disease, according to the district. The oat may therefore be said to have, during its growth cycle, an initial period of high disease potential, an intermediate period of low potential, and later a second period of high potential. It was these well-known changes in what is

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The writer wishes to acknowledge gratefully the help of members of his staff in carrying out the work for this investigation.

now termed disease potential that first led the writer to investigate the possibility of linking them with nutritional changes in the host. Other diseases are known to behave in similar manner. Pasmo disease (caused by *Sphaerella linorum* Wr.) of flax (14) occurs, for example, in early and late phases of the growth cycle, with little or no attack between.

Much work on what may now be called the disease potential of potato to *Phytophthora infestans* at different stages of growth of the host has been published, in addition to that of Beaumont. Pethybridge (15, p. 121) found that younger plants were attacked by blight before older plants, but de Bruijn (6) reported, generally, the opposite result. Beaumont (1) mentions that Pethybridge's "young" potatoes were only 2 or 3 in. high when they were destroyed by the disease, whereas de Bruijn's illustrations show relative behaviour to blight by much older plants. Kühn (11) had indeed suggested that young potato sprouts were very easily attacked by blight, but the plant then attained a period of comparative inability to become diseased, only to be followed in turn by the later and familiar period of high disease potential.

These results, combined with those on blight forecasting mentioned above, suggest that the potato has changes in disease potential through its growth cycle that are similar to those already well recognized on the oat. Confirmation of this important conclusion seemed desirable, so a certain amount of direct inoculation was done on potato in conjunction with the present investigation of host nutrition.

METHODS AND MATERIALS.—*Inoculation.*—Potatoes (*Solanum tuberosum* L.), chiefly Kerr's Pink, at various stages of development, were inoculated on 2 occasions in 1955, 1) in pots within a greenhouse, during February, and 2) in the field during July and August. The inoculum for the first was a suspension containing 25,000 spores of *Phytophthora infestans* per ml, derived from aseptic raw potato-plug cultures of the fungus. For the second, blighted leaves from a first early Epicure crop were suspended among foliage of the late crop, which was not then attacked. Inoculated plants were immediately surrounded by wads of wet filter paper, and each was covered by a bell jar for 56 hours in order to provide the necessary humidity conditions. Records of temperature did not fall below 50°F during either period. When disease appeared on inoculated plants, the identity of the fungus was checked by microscopic examination. The race of the blight fungus was assessed in 1955 by Dr. W. Black of the Scottish Plant Breeding Station, Midlothian, as race 4, or race D as it was originally called by Black.

Analyses of plant material.—"Growth cycle analyses" were carried out on oat (*Avena sativa* L.) and potato. Whole plants were dug with as much root as possible at intervals through the cycle of growth. The plants were carefully washed free of adhering soil, and surface moisture was removed with filter paper. A sufficient number of plants was then divided into roots, stems, leaves, tubers, flowers, and fruits, or as many of these parts as were present. Each sample was weighed, then an appropriate weighed part was dried to constant weight in a steam oven or ventilated dryer and finally ground in a small laboratory hammer mill. Growth cycle analyses were made, as far as possible, on healthy material; in 1950, potatoes were sprayed with Bordeaux mixture to keep the leaves green as long as possible.

Total nitrogen was estimated by means of a centigram-scale Kjeldahl method; ash by incineration in a muffle furnace. Carbohydrate was measured by the picric acid method of Willaman and Davison (17); for total carbohydrate, the weighed sample was hydrolysed with 3 per cent sulphuric acid over steam for 3 hours before the picric acid estimation was applied. With 1 cycle on potato, reducing sugars and soluble nonreducing and insoluble carbohydrate fractions were estimated by the standard methods of Willaman and Davison (17). Percentages of the various fractions in the shoot and in the whole plant were obtained by calculating the weight of each fraction in each component part, adding these figures, and expressing the result as a percentage of the calculated dry weight of the shoot or whole plant.

Disease estimations of potato blight were based upon the British Mycological Society's key (3), whereas measurements of the percentage of leaf spot on oats were made by means of a standard area diagram.

RESULTS.—*Disease potential of the potato for blight.*—The results of inoculations are illustrated in Figure 1; each photograph is typical of the results of 3 inoculations. Young shoots up to 1 in. high were killed rapidly and completely after inoculation with *P. infestans* (Fig. 1, A). At a slightly later stage (3–4 in.) they were defoliated by the disease, but the growing point was not killed (Fig. 1, B). Then came a stage shown by Figure 1, C, when the plant could not be inoculated successfully, and no disease occurred. Rather older plants, however, were susceptible, and the disease developed slowly (Fig. 1, D). Later the crop was attacked by the familiar epidemic. No disease developed at any time on noninoculated controls, or on inoculated plants if suitable climate was not provided, thus confirming earlier work on blight forecasting that a blight epidemic would not start before the necessary weather conditions occurred.

These results confirm Kühn's finding (11) that the potato has an initial period of high disease potential, then a low potential, and finally a second high one. The demonstration of a period of low disease potential also agrees with results of inoculations made by Schilberszky (16). It is not always feasible to portray these changes quantitatively, as no disease usually develops during the initial period of high potential. The semiquantitative expressions used herein, however, indicate adequately the relative disease potential and are explained under Figures 2 and 3.

The growth-cycle analyses.—Six growth-cycle analyses were originally made—1 on oats (variety Star) in 1949, 2 on potato (varieties Arran Pilot and Kerr's

Pink) in 1949, and 3 on potato (varieties Arran Pilot, Dunbar Rover, and Kerr's Pink) in 1950-51. The results of all these detailed analyses are extensive and are not published here in full. Copies are deposited in the library of the British Museum (Natural History), Cromwell Road, London, S.W. 7, together with details of further data obtained in the present investigation. The graphs that appear herewith interpret the data so far as the present problem is concerned.

1) *Potato.*—Results of all 5 potato growth cycles were generally similar, and only 1 of them for Kerr's Pink, 1950-51, is portrayed in full (Fig. 2). This cycle was also examined for carbohydrate fractions. There was a marked fall in percentage of total carbohydrate in the whole plant, the minimum coinciding with the summer period of low disease potential (Fig. 2). High percentages of total carbohydrate were, by contrast, found during disease phases. Arran Pilot and Dunbar Rover, 1950-51, behaved in a manner

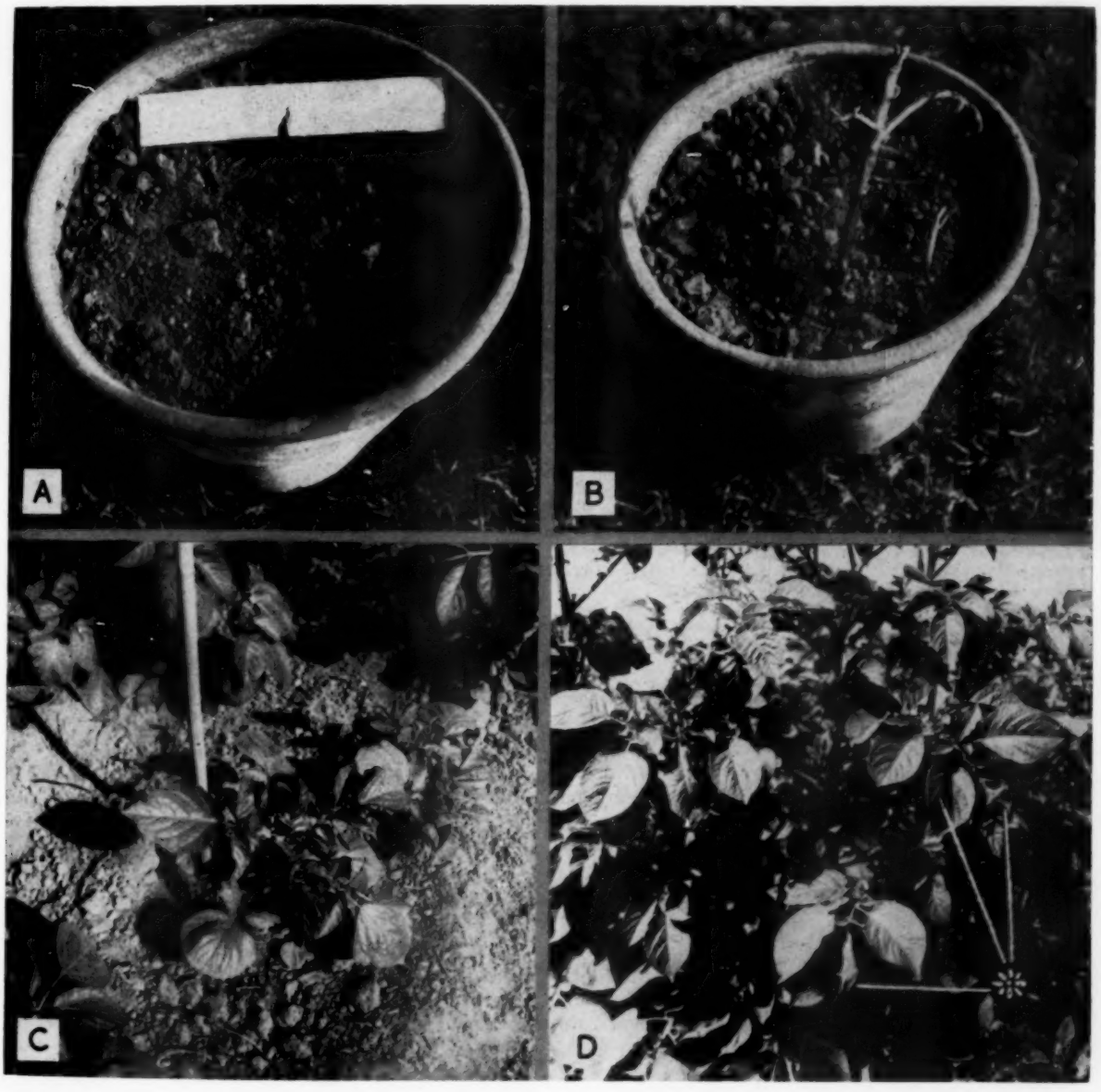

FIG. 1. Typical results of inoculation experiments with *Phytophthora infestans* on potato, variety Kerr's Pink, at different stages of growth. (C_P/R_S ratios were estimated on comparable material.) A) Very high initial disease potential; growing point and sprout killed by blight; ratio, 20.3. B) High initial disease potential, though less than that of A; shoot defoliated by blight but growing point not killed; ratio, 5.41. C) Low disease potential; no blight; ratio, 0.33. D) Moderate disease potential of the later phase; lesions (marked by asterisk) developed slowly on the leaves; ratio, 1.31. A and B were inoculated with spore suspension; C and D with spores from blighted leaves.

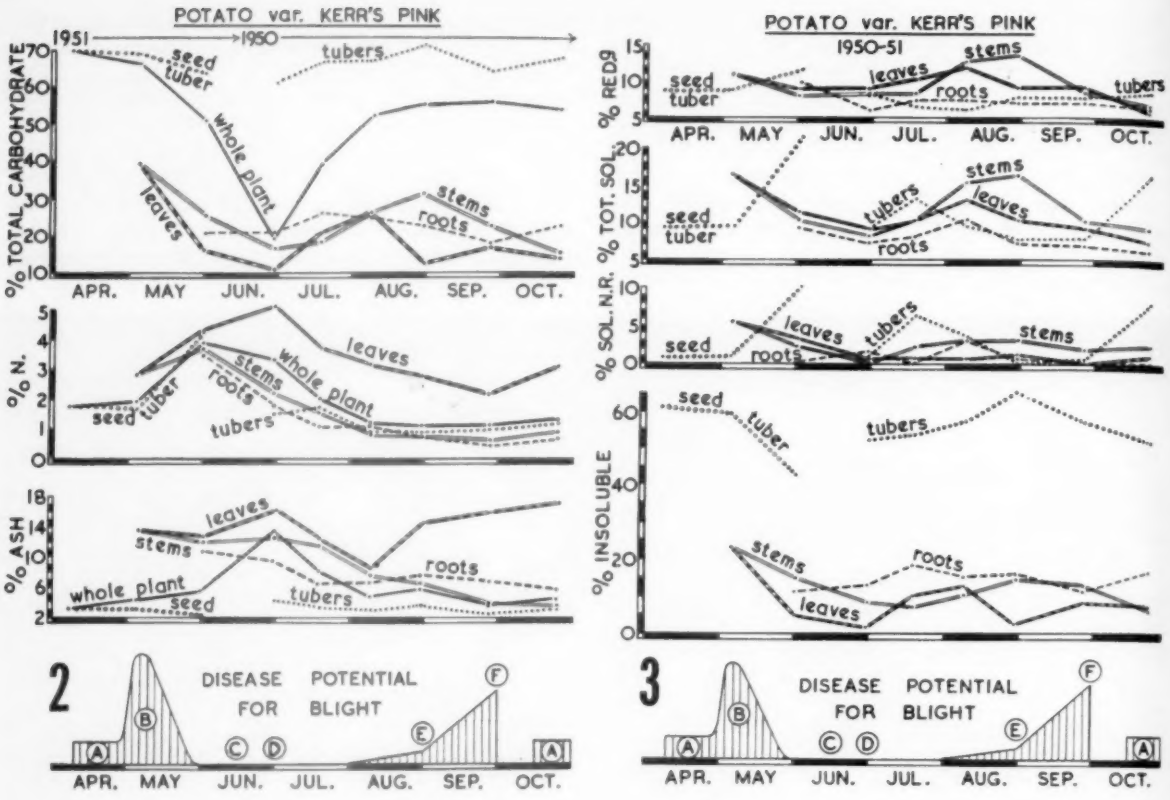

FIG. 2-3. FIG. 2. Results of a growth cycle analysis of potato, variety Kerr's Pink, 1950-51, compared with changes in disease potential for Phytophthora blight. FIG. 3. Variations in carbohydrate fractions (reducing sugars, total soluble carbohydrate, soluble nonreducing sugars, and insoluble carbohydrate) in the growth cycle of potato, variety Kerr's Pink, 1950-51. In both figures, all percentages are of the dry weight. Explanations of the disease potential curves: A) The intermediate potential of the "dormant" tuber. B) Young shoots with the highest potential of all: growing points are killed. C) The time of low potential. D) "Zero time" for blight forecasting in west Scotland. E) The development of the familiar epidemic, plotted quantitatively in relation to F. F) The time of 100 per cent blight on the foliage. F is rated lower than B because growing points are the last to be killed.

essentially similar to Kerr's Pink (Fig. 4). Maxima in the percentage of ash in the whole plant, and in the percentage of nitrogen in the leaves, both coincided with the time of low disease potential in the variety Kerr's Pink (Fig. 2) but not in other varieties (Fig. 4). Carbohydrate fractions (Fig. 3) did not vary in any manner that could be linked effectively with disease behaviour. The period of low disease potential in June coincided with the period of most rapid increase in dry weight (Fig. 5), being generally similar in this respect to the oat.

2) *Oat.*—The percentages, on a dry weight basis, of total carbohydrate, nitrogen, and ash in the various parts throughout the growth cycle are shown in Figure 6. The times of primary and secondary leaf spot disease also are shown, the intervening period being that of healthy growth, i.e. low disease potential. Variations of nitrogen and of ash seemed to bear little relation to the occurrence of leaf spot, but there was a marked fall in percentage of total carbohydrate in the roots during the healthy period. This was reflected in slighter percentage falls in the shoot and in the plant as a whole, and suggested that the oat was relatively low in carbohydrate when the disease potential for leaf spot was low, and *vice versa.* The healthy period was, moreover, a time of rapid increase in dry weight of the plant (Fig. 5).

Consideration of the combined results.—The 2 host-parasite relations investigated agreed in having high percentages of total carbohydrate in the whole plant when disease occurred and low percentages when the hosts were of low epidemic potential, which period also coincided with the time of most rapid increase in dry weight. These 2 factors of carbohydrate and growth rate offered the best correlations with disease behaviour; could an even more definite relation be obtained if they were considered together? A ratio that relates the amount of carbohydrate formed to the amount of shoot that produced it was ultimately found to give a very close and consistent relation with disease potential. This ratio is the weight in grams of total carbohydrate in the whole plant (C_P) divided by the residual dry weight in grams (i.e. dry weight less weight of carbohydrate) of the shoot (R_S) consisting

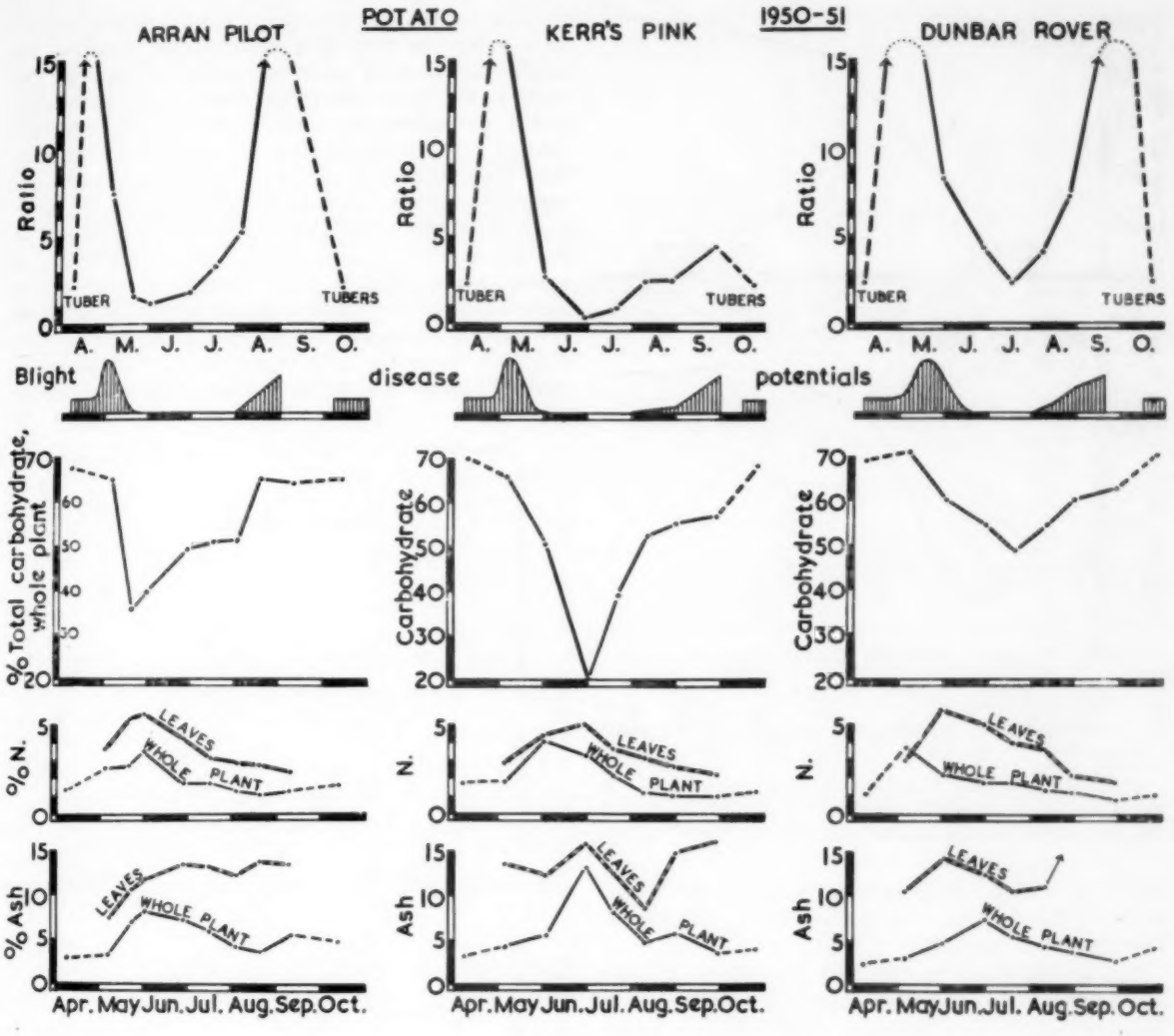

FIG. 4. Variations in C_P/R_S ratio and in content of carbohydrate, nitrogen, and ash throughout the growth cycles of 3 potato varieties. Total carbohydrate, nitrogen, and ash are expressed as percentages of the dry weight, and the percentage of total carbohydrate is that in the whole plant. Conditions in the growing plant are linked to those in the tuber by broken lines.

of both the stem and leaves. For the present purpose this ratio is referred to as the "C_P/R_S ratio," or simply "the ratio."

The oat had high ratios during both disease phases and low ratios in the healthy period (Fig. 7). Ratios in the potato fitted the form of the disease potential diagram far better than did any other factor (Fig. 4). They rose to considerable heights in the sprout and young-plant stages, fell to low levels during the relatively healthy period of June, began to rise after 'zero time' for blight forecasting, and thereafter, the higher the ratio, the quicker the development of blight on the foliage.

The variety Dunbar Rover appeared to have higher ratios throughout than either Arran Pilot or Kerr's Pink (Fig. 4). It also had higher percentages of total carbohydrate during growth. The absolute value of the ratio must evidently be used with care, since the value that separates healthy and disease-prone growth varies for different species and varieties.

Ratios were estimated on healthy noninoculated plants comparable in age to those inoculated with *Phytophthora infestans* as described above, and typical values are included in the descriptions of Figure 1. They also were high when disease potential was high, and *vice versa*.

Changes in C_P/R_S ratios in the tuber or seed.— The "dormant" tuber is a shoot with a high percentage of total carbohydrate and an extremely slow rate of bud growth. Values of the ratio could reasonably be calculated as the weight of total carbohydrate in the tuber divided by its own residual dry weight and

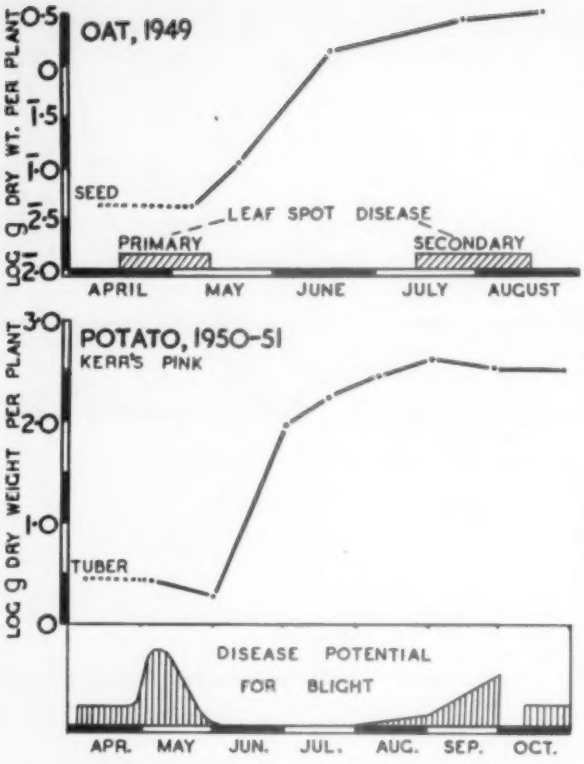

FIG. 5. Growth rates of oat and potato, plotted as log of the grams of dry weight in the whole plant, compared with the respective disease relations.

would inevitably reflect the percentage of total carbohydrate more closely than does the ratio in the actively growing plant.

Tuber ratios did not, in the present work, vary widely from 2.0—an intermediate figure that suggests intermediate disease potential. The value is higher than that of the growing plant during its relatively healthy period and lower than that during the potentially epidemic phases. Development of blight on the tubers was compared directly with that on the foliage during the epidemic of 1954 by lifting tubers in early August and exposing them on the surface to natural inoculation similar to that of the foliage. Results are given in Figure 8, which also shows changes in value of the C_P/R_S ratio. That of the tubers remained fairly steady around 2.0, with relatively slow blight development, whereas that of the foliage rose well above this value and blight development was rapid. "Dormant" tubers are apparently always amenable to this relatively slow development of blight.

When a potato tuber or a relatively large seed like the oat begins to grow, the ratio rises to extremely high values, since the weight of total carbohydrate in the whole plant (tuber or seed plus the new shoot) must then be divided by the residual dry weight of the new shoot. Values as high as 50.4 have been found for sprouting potatoes. Such ratios must be compared with blight relations of the new shoot and not to those of the parent tuber. Young potato sprouts have indeed an extremely high disease potential for blight; the growing point was killed outright at this stage in the inoculation experiments mentioned above, and if, during storage, a patch of tuber blight spreads over an "eye," the latter dies. Seedling blight (primary leaf spot) of oats is also most severe on the earliest stages of growth and causes mortality in the field. Because of this rapid rise and fall in ratio values in the young plant, values in the seed or tuber are, in several figures (Fig. 4, 7, 10) linked with those in the growing plant by broken graph-lines that indicate the general trend but do not fill in all details. The C_P/R_S ratio is the only expression so far used that varies in such a manner as to explain the intermediate

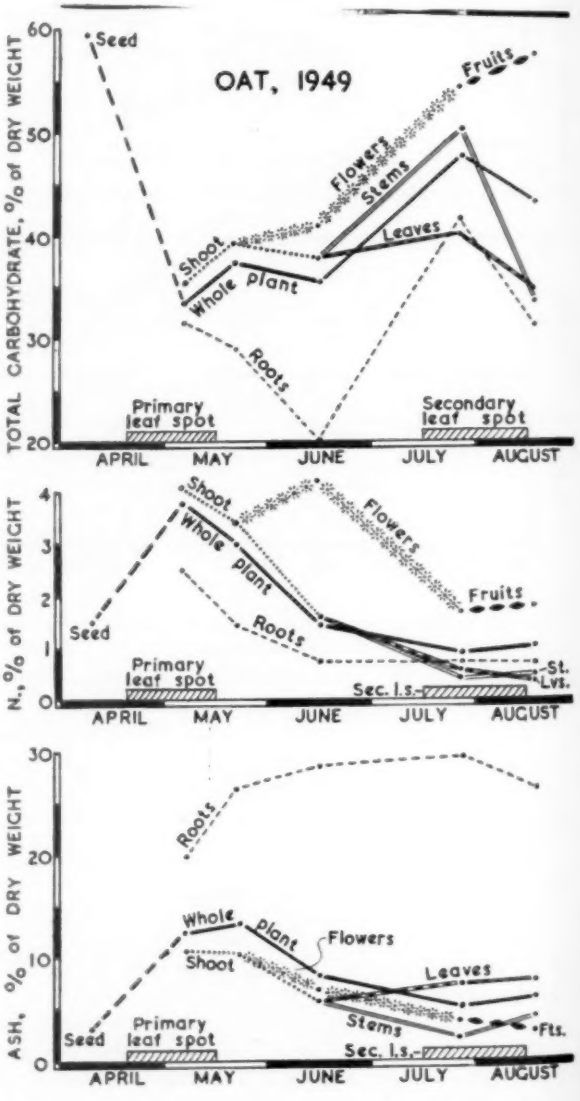

FIG. 6. Results of a growth-cycle analysis of oats, variety Star, compared with the times of occurrence of primary and secondary leaf spot caused by *Helminthosporium avenae*.

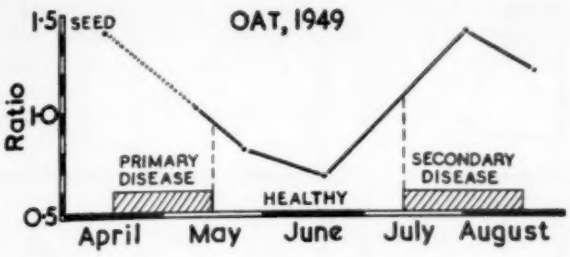

FIG. 7. The relation of C_P/R_S ratio to disease phases and the healthy period of the oat.

disease potential of the "dormant" tuber in addition to all disease behaviour of the growing plant (Fig. 4).

C_P/R_S ratios in potatoes and the rate of epidemic blight development.—Variations in the rate of blight development during the epidemic phase occurred during the years covered by this investigation (Fig. 9). Within a variety, the higher the ratio, the more rapid the development of the epidemic. There is, moreover, over the last 11 years, a strong negative correlation ($r = 0.791$; $P = 0.01$ to 0.001) between the time at which blight first appears and the rate at which the epidemic develops; the later the appearance, the quicker the development. These 2 findings are mutually complementary, for it is almost inevitable that a ratio should rise towards the end of growth. The later the blight appeared, for climatic reasons, the greater the chance of its finding a high ratio in the host, and the more rapid would be its development.

C_P/R_S ratios in potatoes and inherent susceptibility.—Physiological disease potential as defined above, is different from inherent susceptibility or resistance of, say, a plant variety. Ratio values might not therefore have any relation to inherent susecptibility, and it will be shown briefly that they indeed have none. Relative general heights of ratio value would place the 3 varieties mentioned in Figure 4 in order of susceptibility to foliage blight, as 1) Dunbar Rover (most), 2) Arran Pilot, and 3) Kerr's Pink (least). Our own experience over several years would list them 1) Arran Pilot (most), 2) Dunbar Rover, and 3) Kerr's Pink (least). Table 1 shows that ratio values were not related to the innate relative tuber susceptibility of the variety.

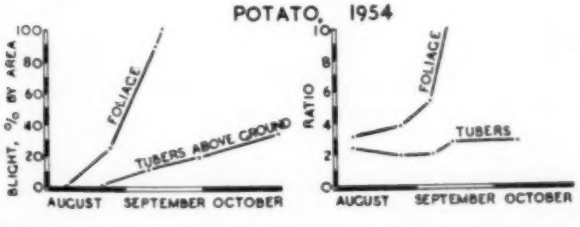

FIG. 8. Relative rates of development of blight on potato foliage and on tubers aboveground compared with respective values of the C_P/R_S ratio (variety Kerr's Pink, 1954).

TABLE 1.—*C_P/R_S ratios and relative tuber susceptibility to blight*

Variety	Blight on tubers (Percentage by weight[a])	C_P/R_S ratio of the tubers[b] 1955	1954	1950	1949
Dunbar Rover	24.8	2.49		1.82	
Gladstone	20.4	1.90			
Arran Pilot	16.9	2.51		1.94	2.07
King Edward	16.4	2.11			
Kerr's Pink	13.2	2.22	2.17	1.84	2.20
Golden Wonder	1.2	2.17			
Orion	(Resistant)	2.42			

[a] Unweighted percentage mean values, 1945-55, except for Golden Wonder which is the mean for 1954 and 1955. Tubers of the variety Orion were obtained from Northumberland in 1955. All the remaining data were obtained at Auchincruive.

[b] Estimated in September in each year.

Tests of the relationship between C_P/R_S ratio values and disease potential.—By growing crops at different times of the year, it is possible to test whether ratio values are merely coincidental or are related to disease behaviour. The very uniformity of pattern in ratio changes with age should then make it possible to have plants with high values growing alongside others with low ratios. If disease merely occurs at particular times without relation to ratio values, it should occur at those times on all crops, no matter what their stage of development or when they were planted. All the plants at any time should be healthy, or all should be attacked to the same extent by the disease. If, however, there is a relation between ratios and disease behaviour, the parasite should attack most when ratios are high in the host and least when they are low. This would mean that, given evidence of continuing infection, healthy plants would be growing alongside diseased ones, or at least that crops with serious infections would be close to hosts having but little disease.

1) *Oat.*—The sowing of oats at different times of the year would provide continuing natural infection, as there would be a succession of primary disease phases followed by a succession of secondary occurrences. In Figure 10, changes in ratio values are compared with the amounts of Helminthosporium leaf spot on 6 neighbouring strips of oats (variety Star) sown at different times in 1955. Plants with disease did indeed occur close to healthy ones at the same time, and at all times the disease potential was closely related to height of the C_P/R_S ratio. Primary leaf spot was estimated by growing seed in pans of sand, for many infected seedlings die under field conditions as mentioned above. Slight primary infection, up to 0.1 per cent, could, however, be seen on the field crops for a period (Fig. 10). Secondary leaf spot was estimated as the percentage of total leaf area, so that 5 per cent is quite a noticeable infection. The amounts of disease were recorded as they occurred,

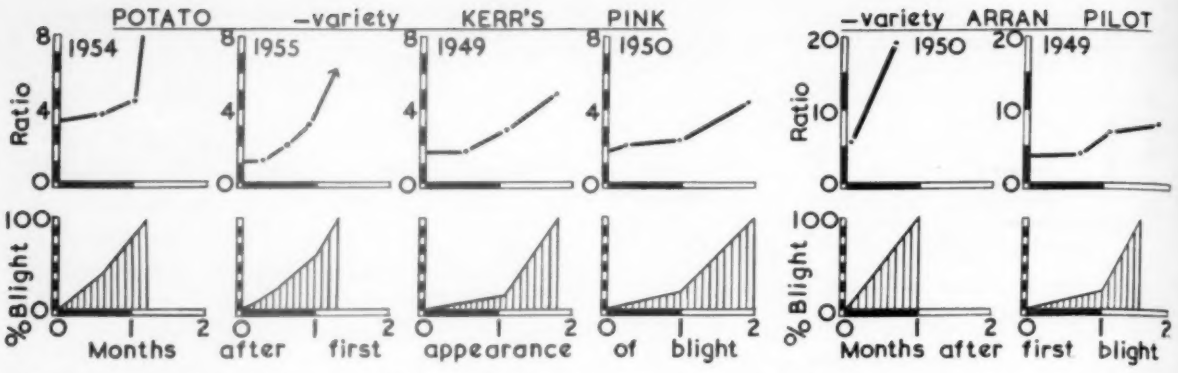

FIG. 9. The rate of development of the blight epidemic (shaded) in 2 potato varieties compared with the pattern and height of the C_P/R_S ratio curve in different years.

and it was not until the ratio values were added later that the close relation between them and disease potential was seen. Indeed, the healthy period of low disease potential coincided well with the time during which the ratio curve fell below 0.5 in this particular year (Fig. 10).

This result also shows that the healthy periods are caused by conditions within the host and not by lack of inoculum, since this is constantly provided by neighbouring plants.

2) *Potato.*—Tubers of the variety Kerr's Pink were planted at approximately monthly intervals from April to July in 1954 and from April to August in 1955. Disease relations of this crop differ from those of the oat in that 1) the early period of high disease potential is not normally manifested by actual disease, and 2) a period of suitable climate must occur before the usual epidemic attack can begin at the second phase of high potential. It is nevertheless possible to compare the rate of blight development with ratio values during the natural infection of an epidemic when it occurs (Table 2, Fig. 11, 12).

Plants with severe disease and high ratios grew next to plants with much less disease and low ratios (Fig. 11). Blight developed on the foliage earlier and quicker on the normal crop with higher ratios than it did on the later-planted crops with lower ratios; even the tubers appeared to follow this relation (Table 2). The restriction on the time of first appearance of blight imposed by the need for adequate climate made it possible for the ratio value to be quite high when the disease first appeared on the normal crop (Fig. 12, upper diagram). When this happened, the epidemic developed quickly, and rising ratios brought increased disease potential.

By planting tubers sufficiently late in the year, it should be possible to arrange for shoots on plants with the very high initial ratio values to be emerging during the blight epidemic. This first period of high disease potential was assessed on a crop from tubers planted August 26, 1955 (Fig. 12, lower diagram). Ratios were falling from high values when blight first appeared, and the disease developed very rapidly indeed, up to September 20, when 50 per cent of the shoots and their growing points were killed, and there was 75 per cent defoliation by blight on the whole crop. By September 23, however, the ratio had fallen to a relatively low level, and thereafter blight development was slow on such shoots as had survived the initial destruction. Disease potential still reflected ratio height in this special case where a plant with falling ratios tends to "grow out of" the disease.

Nitrogen and ash were determined for the normal plantings and for the latest plantings in 1954. Variations in these fractions were such as would be expected of the more juvenile development of the late-

TABLE 2.—*Potato blight and C_P/R_S ratios, 1954*

Date of analysis or disease estimate	Ratios or disease estimation on crops planted on indicated dates			
	April 10	May 6	June 8	July 3
	C_P/R_S ratio			
July 6	0.89	0.30	0.70	0.76
Aug. 6	3.10	1.44	0.78	0.52
Aug. 24	3.74	2.77	1.62	0.78
Sep. 6	4.37	4.81	3.07	1.45
Sep. 14	11.3	4.61	3.36	1.34
Sep. 24		6.11	5.46	1.58
Oct. 8				5.10
	Blight on foliage[a] (percentage of area affected)			
Aug. 21	35	25	10	4
Aug. 30	50	30	12	5
Sep. 6	80	50	25	10
Sep. 13	95	80	40	20
Sep. 22	100	93	75	35
Sep. 28	100	100	97	60
Oct. 4	100	100	100	95
Oct. 8	100	100	100	100
	Blight on tubers (percentage of area affected)			
Aug. 6	0	0	0	0
Aug. 24	0.01	0	0	0
Sep. 6	0.02	0.015	0.013	0
Sep. 13	0.05	0.01	0.04	0
Sep. 24	0.5	0.2	0.2	0.02
Oct. 8				2.1

[a] On the crops planted April 10, May 6, June 8, and July 3, blight first appeared on the foliage on Aug. 6, Aug. 8, Aug. 12 and Aug. 19, respectively.

planted crop as compared with the normal one, and as such would be computed by the ratio. The ratio of water to nitrogen suggested by Collins (5) also was compared with disease potential; it was fairly satisfactory during the second phase of high potential, but did not apply for the first phase.

Statistical significance of results.—The results of statistical analyses of the data are given in Table 3. Percentages of total carbohydrate in the whole plant were here related to the amount of disease almost as

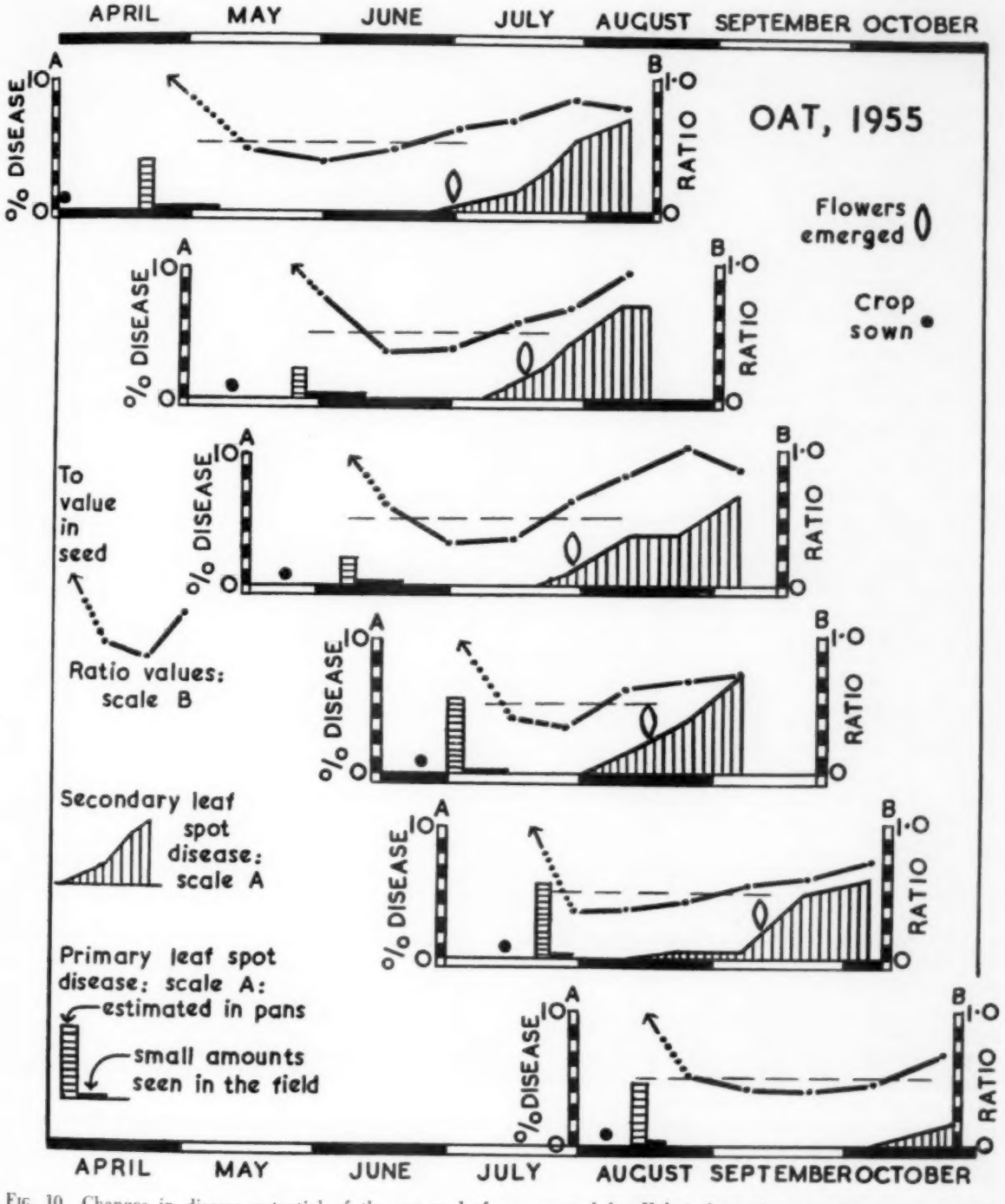

FIG. 10. Changes in disease potential of the oat to leaf-spot, caused by *Helminthosporium avenae*, compared with changes in C_F/R_S ratio for crops grown at different times of the year. The period of health or low disease potential corresponds to the time when the ratio falls below the value (0.5) denoted by the broken line.

TABLE 3.—*Statistical significance of correlations with various factors*

Crop, disease, year, and item correlated with percentage of disease on foliage	Value of correlation coefficient[a] r	Range of P by t-test	Number of pairs
Potato, Phytophthora blight, 1954			
C_P/R_S ratio	+0.773**	0.001	22
Percentage of total carbohydrate in the whole plant	+0.664**	0.001	22
Percentage of nitrogen in the whole plant	−0.592*	0.1–0.05	9
Percentage of ash in the whole plant	−0.556	0.1	9
Water/nitrogen ratio in the shoots[b]	+0.850**	0.01–0.001	9[c]
Water/nitrogen ratio in the leaves[b]	+0.350	0.1	9[c]
Potato, Phytophthora blight, 1955			
C_P/R_S ratio	+0.827	0.05–0.02	6[d]
Percentage of total carbohydrate in the whole plant	+0.850*	0.05–0.02	6[d]
Oat, Helminthosporium leaf spot, 1955			
C_P/R_S ratio	+0.733**	0.001	39
Percentage of total carbohydrate in the whole plant	+0.730**	0.001	39

[a] * Indicates significance at the 5 per cent level; ** at the 1 per cent level.
[b] See Collins (5).
[c] These correlations are valid only when applied to the later phase of disease. They become less significant or not significant in proportion to the amount of data included from the first disease phase.
[d] Correlations for a representative cross section of determinations (see text).

significantly as was the C_P/R_S ratio, though the ratio has been shown to fit the whole disease potential curve better than any other factor (Fig. 4). Calculations of the correlation coefficient over the whole data are, however, sometimes open to certain objections. One such objection was that in 1955, when blight appeared very late, 49 determinations of ratio value were made but there was no disease with which to correlate most of them. In order to avoid a personal selection, correlations were calculated from the small cross section of results specified in Figure 11; these were significant at the 5 per cent level. Dr. D. J. Finney, of the Agricultural Research Council's Unit of Statistics, Aberdeen, suggested an alternative test for the oat data of Figure 10. It is based upon the probability of overlap between the period of low ratio (i.e. less than 0.5) and the period of low disease potential. The result confirms that the association is statistically significant at approximately the 1 per cent level.

Late planting is not a practical way of minimizing attack by blight or leaf spot, for the yield of tubers or oat grain is never so large as that which follows normal planting. The experimental demonstration, on 2 crops and in 2 years, of such close relations between ratio and disease, however, supports the main thesis of this paper. A physiological balance within the host has been shown to affect its disease potential for a fungal parasite.

DISCUSSION.—This investigation of changes in the major constituents of a plant host in relation to its disease potential towards a fungus has indicated that carbohydrate status in the whole plant was the most important of any of the nutrient fractions estimated for 2 diseases. The relation became more precise when carbohydrate was considered in relation to general growth by the calculation of the C_P/R_S ratio, for this ratio is as much an indication of stage in plant development as of carbohydrate status. It does, in fact, integrate many factors and is, moreover, a far wider concept than was thought possible when this investigation was begun.

Ratio values appear to explain all the variations in disease potential through the growth cycle. One of the primary objects of the present work was to find a possible explanation for the need for a "zero time" in blight forecasting. This is now shown to mark the end of a period of low disease potential corresponding to the time of lowest values of the C_P/R_S ratio in the host. Differences in the disease potentials of actively growing plants and "dormant" tubers, the rate of development of the yearly epidemic, and the occurrence of a healthy or low-disease period between primary and secondary phases of disease are all referable to ratio values. Such values do not, however, explain the relative inherent susceptibility of varieties, which is apparently governed by different factors. Neither would they explain "mature plant resistance" as suggested for certain cereal rust diseases. Ratio values have explained several problems relating to disease, but they must still be considered along with other factors, such as climate, that are also involved.

Ratio values that demarcate healthy and diseased conditions of the growing plant varied from 0.5 to 1.0 in the oat, were approximately 1.0 in Kerr's Pink potato, about 2.0 in Arran Pilot potato, and probably lie somewhere between 4 and 5 for Dunbar Rover. The ratio is therefore relative rather than specific; has it any direct practical value? Ratios may rise considerably if there is any check to growth, and demarcation values may vary with differing climates of different years (cf. the oat in 1949 and 1955). Ratios have, moreover, been known to rise and then fall again during July, as with May-planted Kerr's Pink potatoes in 1955. It would seem that the ratio value can vary, and may presumably be varied, from its overall pattern of change, in sufficient degree to warrant the conclusion that further study of factors that influence it should lead to results of direct practical significance.

Though the C_P/R_S ratio has been found to correlate well with disease behaviour, there is at present no clear indication as to how such a broad physiological balance within the plant can exert the effect it does.

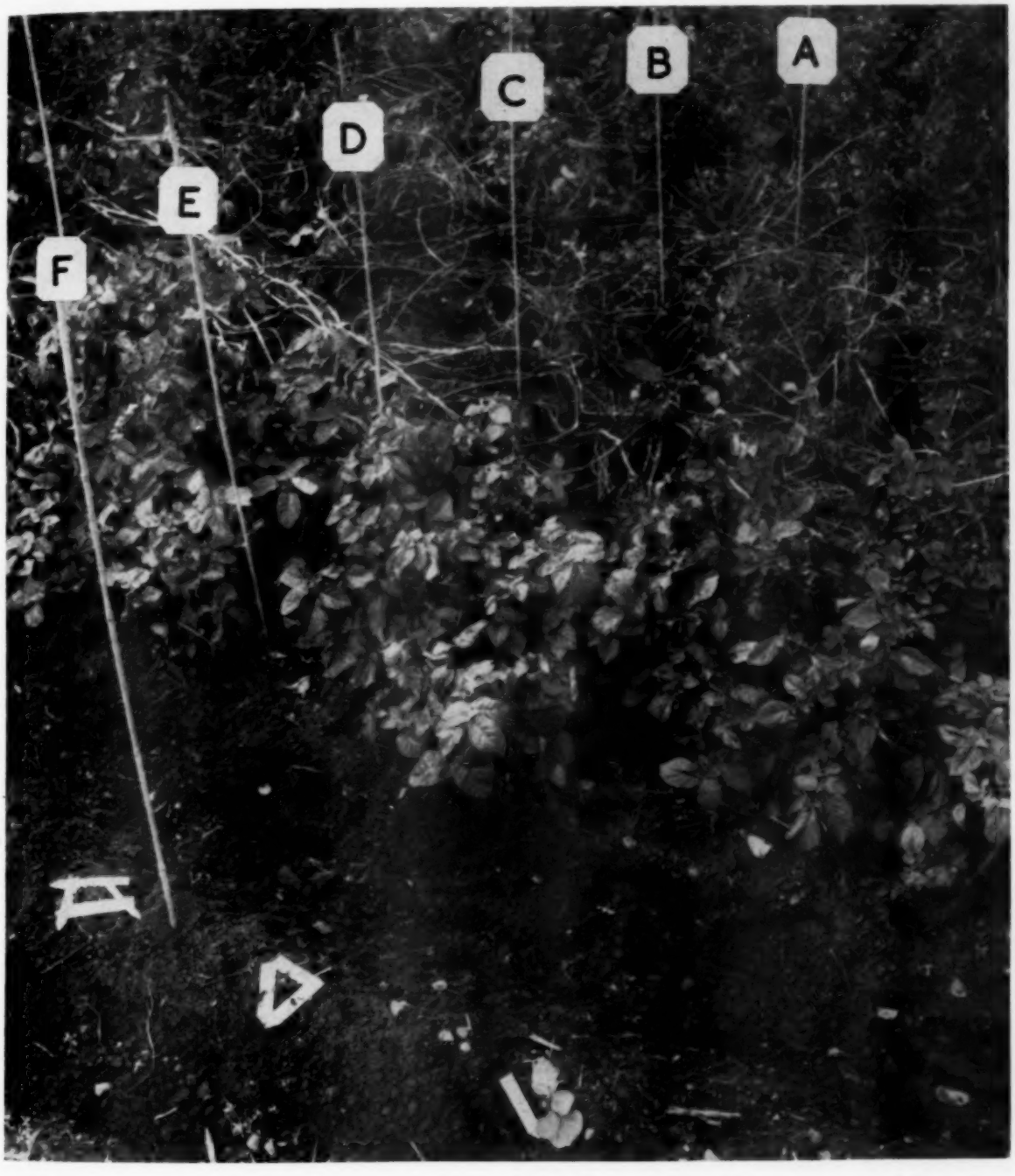

FIG. 11.—Potatoes planted at different dates and photographed on October 8, 1955. A) Planted April 18, 95 per cent of foliage blighted at time of photograph, C_P/R_S ratio = 2.98. B) Planted May 10, 90 per cent blighted, ratio = 1.92. C) Planted June 17, 75 per cent blighted, ratio 1.24. D) Planted July 14, 25 per cent blighted, ratio = 0.90. E) Planted August 5, 30 per cent blighted, ratio = 1.03. F) Planted August 26, 95 per cent blighted, ratio = 1.90. A–C) Rising ratios during second phase of high disease potential. D–E) Low ratios, low disease potential. F) Initial phase of high potential: most shoots and growing points were killed entirely between September 9 and September 23, during which the ratio fell from 11.6 to 1.61. One of the few (5 per cent) shoots that survived is evident in the centre foreground. Dead shoots can be seen against the triangle of white labels.

An investigation of its mechanism of action is an inevitable future study. The ratio hypothesis seems to have most in common with the known effects of soil nutrients on disease severity, for they can affect growth, which is computed in the ratio. Growth of many fungi is accelerated by the addition of various soluble carbohydrates to the culture medium, so the action of a ratio might be investigated further along such a direct line of approach. No evidence was found in the present investigation, however, that soluble carbohydrate was related to disease behaviour; the carbohydrate relation was with the total fraction. There are difficulties as yet about linking the ratio hypothesis with thickness of cuticle (4), with osmotic pressure of host tissues (4), or with the production of specific substances (13) or fractions (10); only further work can elucidate this matter.

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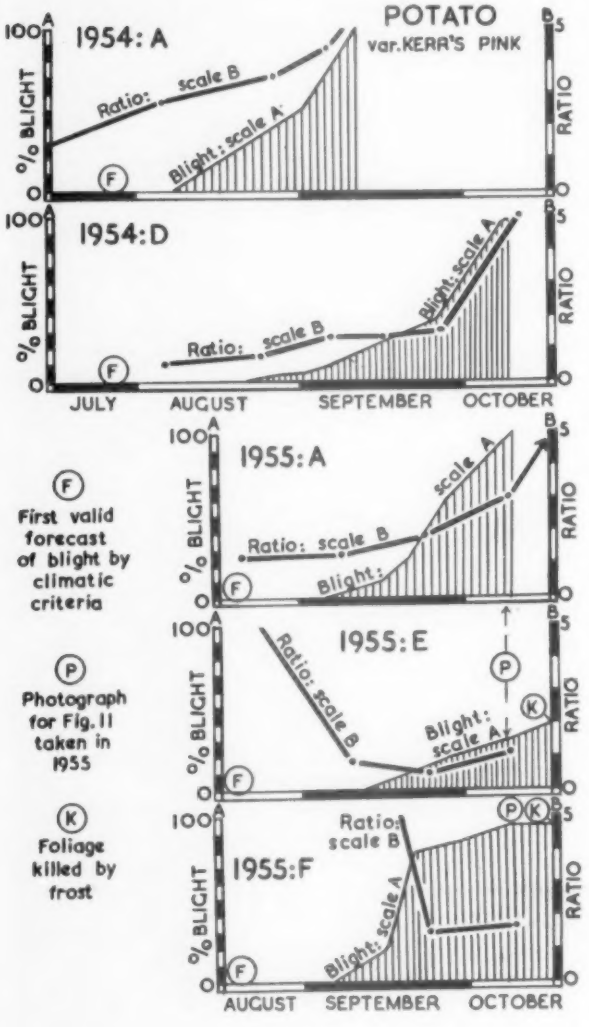

FIG. 12. Changes in C_P/R_S ratio values compared with blight development on the foliage of potato crops planted at different times of the year. 1954:A, planted normally on April 10, has high ratios and high disease potential for blight of the later phase, whilst, at the same time, 1954:D, planted July 3, has low ratio values and low disease potential. 1955:A, planted normally on April 18, has rising ratio values and high disease potential of the later phase, whilst, at the same time, 1955:E, planted Aug. 5, has low ratios and low disease potential, and 1955:F, planted Aug. 26, has high ratios and high potential of the initial phase followed by low ratios and low potential.

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DETERIORATION OF BARLEY IN STORAGE BY MICROORGANISMS[1]

N. Armolik, J. G. Dickson, and A. D. Dickson[2]

SUMMARY

The storage molds contributed to the loss of germinability of barley stored at high moisture content. Storage under limited aeration reduced germination rapidly. *Fusarium moniliforme* appeared to reduce the germinability more rapidly than did species of *Aspergillus* and *Penicillium*. The filtrates from cultures of *F. moniliforme* and *A. niger* reduced germination of barley.

Barley varieties grown at several locations in the United States behaved differently in storage with high moisture. The small-kerneled actively germinating varieties were damaged more than the large-kerneled types with slower rate of germination. Both variety and environment under which the barley matured were important factors in determining tolerance to poor storage conditions. The samples from Aberdeen, Idaho, which were harvested under dry conditions, generally were more tolerant than those from Madison, Wisconsin, where environmental conditions rarely favor full development of kernel dormancy, especially of combine-harvested grain.

The deterioration of barley during storage by the activity of microorganisms has been a problem of economic importance, especially in the use of barley for malting and for seed. Within the past 5 years, the malting industry has had to sell for feed many bushels of barley because of the loss of germinability in storage. Numerous articles have been published concerning the storage of wheat, corn, soybeans, rice, etc. In a recent monograph published by the American Association of Cereal Chemists (1), the important papers are summarized. Little attention has been given to the storage of barley.

The present study was initiated in an attempt to learn certain basic facts in regard to the role of storage molds in the deterioration process. The emphasis was placed on germinability of the barley during and after storage, since the greatest damage to malting barley has occurred during the early storage period.

MATERIALS AND METHODS. — Barley samples were studied from 4 areas differing in environmental conditions during maturation of the grain. Barleys were grown in experimental plots in Aberdeen, Idaho; Bozeman, Montana; Davis, California; and Madison, Wisconsin. At the several locations, wide differences occurred in maturity of the grain and activity of the embryo, the moisture content of the grain when harvested, and the microflora on the kernels. All samples were plated, with and without surface sterilization, initially and after storage to determine the fungi and bacteria present.

Barley samples of 200 g each were surface sterilized 1 minute in a mixture of 40 per cent ethyl alcohol and 2.5 per cent sodium hypochlorite, rinsed in sterile water, and dried 24 hours in a dry room between sterile paper towels. The moisture content of the seeds was determined, and the amount of water required to bring the seeds to the desired moisture level was added. After distribution of the moisture, the samples were divided aseptically in 2 lots and placed in parallel storage containers. The experiments were replicated and frequently were repeated when analysis of the data indicated the need.

The storage experiments were conducted at 2 moisture contents, 18 and 25 per cent. One series of samples, placed in glass tubes, was aerated continuously with moisture-conditioned air, while the other series, stored in 1-quart jars with screw caps fitted with rubber gaskets, was aerated at limited periods. Distilled water or saturated Na_2CO_3 solution was used for humidification of air.

The germinability of the seeds was determined by placing 100 kernels on moist paper blotters and allowing them to germinate at 16°C. The germination readings were taken after 7 days.

The fungus invasion was determined by plating the kernels, after surface sterilization, on culture media. As the composition of the medium influenced the kinds of fungi isolated from seed (2), 2 media were used. These were malt-salt agar (3), a medium especially suited for detection of storage molds, and potato-glucose agar for detection of bacteria and other fungi.

For testing the toxicity of culture filtrates, the fungi were grown at 22°C in shake cultures in 250-ml Erlenmeyer flasks containing 80 ml of Richard's solution. The mycelial mats were removed and the filtrate sterilized by autoclaving. The seeds were soaked in the filtrates for 24 hours at 16°C, then removed and germinated on moist paper blotters at 11°C.

EXPERIMENTAL RESULTS. — *Studies with individual*

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Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Field Crops Research Branch, Agricultural Research Service, United States Department of Agriculture. The research was supported in part by an Industrial Research Grant from the Barley and Malt Institute, Chicago, Illinois. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Credit is given to Eugene Herrling for illustration preparation. Dr. K. B. Raper checked the identification of the *Aspergillus* and *Penicillium* species.

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mold species.—Numerous articles dealing with fungus species isolated from the deteriorated grain under particular conditions have been reported (2, 4, 6, 7); however, the effect of individual mold species on seed germinability is little known.

Six species of fungi, namely *Fusarium moniliforme* Sheldon, *Aspergillus flavus* Lk., *A. niger* v. Tiegh., *A. amstelodami* (Mang.) Thom & Church, *Penicillium cyclopium* Westling, and *P. chrysogenum* Thom, were isolated from deteriorated barley and used for inoculation of surface-sterilized Atlas barley. Atlas barley grown in 1953 at Davis, California, was used as it was more resistant to storage damage at high moisture than other varieties studied, and it was free from storage molds. Samples with adjusted moisture contents of 25 and 18 per cent were stored with continuous and with limited aeration. Small samples from the tubes and from the sealed jars were removed after 5- or 10-day intervals and tested for germinability and for the location of the fungus, i.e. whether on the surface of the kernels or in the embryos. Storage under limited aeration resulted in relatively low oxygen and high carbon dioxide content at the end of each 5- or 10-day storage interval.

The germination of Atlas barley was reduced appreciably by conditions of limited aeration and by specific fungi (Fig. 1, A). The germination of barley stored at 25 per cent moisture under limited aeration was reduced rapidly without heating or without the development of moldy kernels. Both *F. moniliforme* and *P. chrysogenum* reduced germination under both limited aeration and aerated storage (Fig. 1, A, B). The results were different perhaps than those reported by Peterson et al (5) who reported that high concentrations of carbon dioxide inhibited fungus growth in grain.

The barleys stored at 18 per cent moisture responded similarly to those at 25 per cent; however, the rate of loss in germinability was slower in the former. The lower moisture retarded the rate of kernel invasion by the fungi. *Alternaria* spp. originally present on the kernels increased during the period of storage. An acid-tolerant white bacterium increased in some barley lots.

Varietal and locational response to high moisture storage.— Experiments showed that different barley samples differed considerably in the rate of deterioration when stored at high moistures. Although germination was similar originally, the Manchurian type of barleys appeared more sensitive to poor storage con-

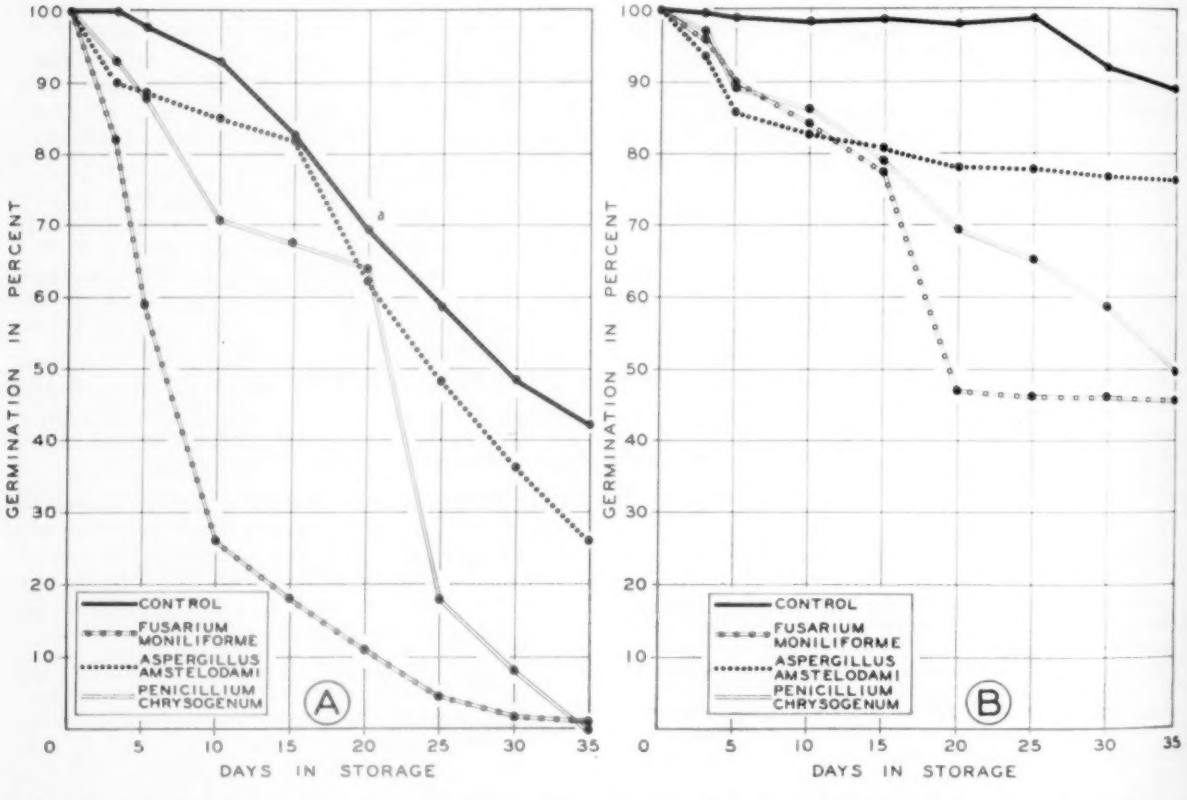

FIG. 1. Graphic summary showing average percentage germination of Atlas barley as influenced by storage at 25 per cent moisture under both limited and continuous aeration and in the presence of storage fungi. A) Barley stored under limited aeration. B) Barley stored with continuous aeration. *Aspergillus amstelodami* started development in the controls at the point indicated by (a).

TABLE 1.—*Average percentage germination of several barley varieties from seed harvested in different localities and stored at 25 per cent moisture with continuous aeration*

Variety and origin	Percentage germination before storage	Average percentage germination after indicated storage period			Organisms that increased during storage
		3 Days	6 Days	10 Days	
Kindred, Aberdeen 1952	100	76	51	70	Alternaria
Moore, Aberdeen 1952	99	55	37	21	Alternaria, Bacteria
Montcalm, Aberdeen 1952	99	96	84	57	Alternaria
Kindred, Aberdeen 1954	100	91	59	39	Alternaria
Hannchen, Bozeman 1954	100	96	91	72	*Fusarium culmorum*, Alternaria, Bacteria
Betzes, Bozeman 1954	99	93	98	92	Alternaria, Bacteria
Ymer, Bozeman 1954	100	94	94	82	*F. culmorum*, Bacteria
Kindred, Madison 1954	95	26	10	1	Alternaria
Moore, Madison 1954	98	65	51	21	Alternaria
Montcalm, Madison 1954	97	60	38	12	Alternaria
Atlas, Davis 1953	100	99	99	98	Alternaria

ditions than did the North African and the 2-rowed types. Seven barley varieties grown in 1 or more of 4 localities in the United States were compared under comparable storage conditions. The varieties Kindred, Moore, and Montcalm grown in 1952 in Aberdeen, Idaho, were compared with the same varieties grown at Madison, Wisconsin, in 1954. Tests were made also with three 2-rowed barley varieties, Hannchen, Betzes, and Ymer, grown at Bozeman, Montana, 1954; with Kindred barley grown at Aberdeen in 1954, and with Atlas barley grown at Davis, California, in 1953.

The barley samples were prepared, adjusted to a moisture of 25 per cent, and stored with continuous aeration as described. The germination of the kernels and the microorganisms present were determined after 3, 6, and 10 days of storage.

The loss of germination under storage at 25 per cent moisture was pronounced; it was influenced by both variety and location where grown (Table 1). The percentage germination of Kindred, Moore, and Montcalm, varieties characterized by small kernels and rapid germination, was reduced rapidly in the samples originating both at Aberdeen and at Madison. The varieties from Aberdeen generally were damaged less by poor storage conditions than were those from Madison. Atlas and the 2-rowed varieties Hannchen, Betzes, and Ymer were damaged less by high-moisture storage than were the small-kerneled varieties. The difference in response probably was associated with differences in degree of dormancy and in the physiological response of the embryos of the several contrasting types of barley.

The effect of autoclaved culture filtrates of mold fungi on germination of barley.—The role of molds in grain deterioration was investigated further by the use of filtrates prepared from cultures of the fungi. Kindred barley grown in 1954 at Aberdeen, Idaho, was used for testing the effect of filtrates.

Isolates of *Fusarium moniliforme, Aspergillus flavus, A. niger, A. repens, A. amstelodami, A. ruber, Penicillium cyclopium,* and *P. chrysogenum* were compared for toxic action on germination. The fungi were grown in triplicate cultures on Richard's solution as described, filtered, and autoclaved. The solutions were prepared after 5, 10, 20, 30, 40, and 60 days' growth of the fungi. Barley steeped in Richard's medium was used as a control.

Culture filtrates from 6 of the fungi reduced germination significantly when compared with the control (Table 2). The toxicity of the culture filtrates varied somewhat with period of growth of the fungi on the Richard's solution. The maximum toxicity of the filtrates from *F. moniliforme* and *P. chrysogenum* developed early, after 10 days' growth; that of *A.*

TABLE 2.—*Germination of Kindred barley after 24 hours steep in autoclaved filtrates of Fusarium, Aspergillus, and Penicillium cultures grown for 5 to 60 days on Richard's solution*

Age of culture (days)	Percentage germination of control[b]	Percentage germination[a] after steep in indicated culture filtrate							
		F. moniliforme	*A. flavus*	*A. niger*	*A. repens*	*A. amstelod*	*A. ruber*	*P. cyclopium*	*P. chrysogenum*
5	91	72	91	86	92	91	90	85	87
10	99	55	87	76	94	94	91	86	83
20	93	67	93	47	91	93	85	84	93
30	93	65	90	52	92	91	83	82	89
40	96	86	85	84	90	91	91	87	86
60	95	85	93	90	96	97	97	90	93
Average	93	72	90	72	92	93	90	86	88

[a] LSD. (5 per cent) = 3.
[b] Steeped in Richard's solution.

niger, *A. ruber*, and *P. cyclopium* after 20–30 days. The filtrates from the older cultures of each of the fungi did not reduce greatly the germination of barley.

All of the culture filtrates, except those from *P. chrysogenum*, affected early seedling development (Fig. 2). The development of both root and coleoptile were affected by most filtrates. The influence of the filtrates of *F. moniliforme* on germination and seedling development is given in Table 3. Maximum effect in reducing germination in the filtrates was from the 10-day-old cultures. Abnormal seedlings with slender elongated shoots, with fine rootlets without root hairs, and with initials of fine roots that ruptured the coleorhiza without further elongation developed from barley steeped in filtrates from 20- to 30-day-old cultures. The filtrates from the other fungi reduced vigor of germination and seedling development.

Transfer of steeped kernels to fine silt loam in the greenhouse decreased the effect of the filtrate upon both germination and seedling growth. When this was done, filtrates from 20-day-old cultures of *A. niger* reduced germination to 64 per cent in contrast to 47 per cent in tests made on filter paper. Steeping in filtrates from *F. moniliforme*, while not affecting germination greatly, resulted in elongated, slender seedlings.

Discussion.—The preservation of the original high quality, germinability, and vigor of embryo development has been the major objective in the storage of barley in large volume for use in malting and for seed. During the transition period from old methods of threshing to the combine harvesting of barley, especially in the humid and semihumid barley production areas, experience has indicated the need for modification of storage conditions and for the use of different criteria for insuring safe storage conditions. Large quantities of barley containing apparently a safe moisture content for storage have given low germination 6 months after they were placed in the elevators. Generally this change in germination capacity has occurred without heating or any of the characteristic reactions associated with grain spoilage in storage.

The relation of storage molds to this change in germination capacity, discussed in the present paper, represents 1 phase of the investigations in handling and storage of barley. The fungi selected for study were prevalent in barleys from farmers' bins during

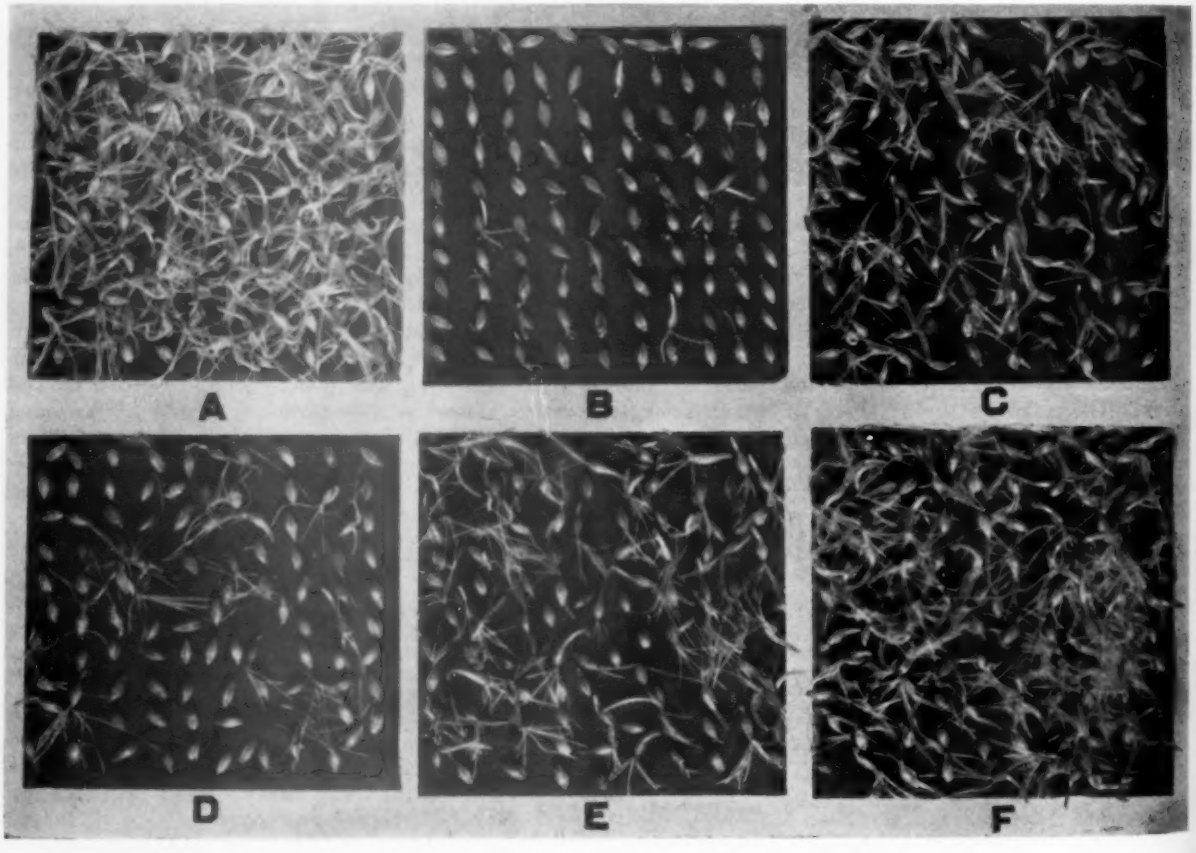

Fig. 2.—The effect of 20-day-old culture filtrates of several fungi on seed germination and seedling development. Barley kernels were steeped 24 hours in autoclaved filtrates. A) Control (Richard's medium). B) *Fusarium moniliforme*. C) *Aspergillus flavus*. D) *A. niger*. E) *Penicillium cyclopium*. F) *P. chrysogenum*.

TABLE 3.—*Percentage germination and percentage of abnormal seedlings that developed following treatment of barley seed with culture filtrates of Fusarium moniliforme*

Age of culture (days)	Percentage germination	Percentage of abnormal seedlings
5	72	11
10	55	16
20	67	57
30	65	47
40	86	25
60	85	7

relatively short periods of storage after harvest. With the exception of *F. moniliforme*, they are reported as common storage molds in other grains.

The moisture contents of 18 and 25 per cent were selected primarily because barley transferred from the combine to farmers' storage bins frequently was in this moisture range, and because fungus development and physiological changes progressed rapidly at these moisture levels.

Several of the fungi, notably *F. moniliforme* and *Penicillium* species, reduced appreciably the germination of barley in a 10-day storage period, especially under limited oxygen supply. Low oxygen supply or perhaps the increased carbon dioxide content during storage in the absence of much fungus development reduced germination rapidly as illustrated in the control samples; however, Peterson et al (5), using wheat at 18 per cent moisture and a 10°C higher storage temperature, showed that carbon dioxide concentration of 40 per cent and above protected viability. With continuous aeration of the samples, the mold growth was more conspicuous over the kernels than was the case under reduced aeration, but the reduction in germination was less and progressed more slowly.

Based on preliminary evidence, barley varieties responded differently to the storage conditions used. The Manchurian-like varieties characterized by small kernels, rapid rate of water absorption, and active germination (Kindred, Moore, and Montcalm) were damaged quickly. In contrast, the Atlas and 2-rowed varieties with large kernels and slow rate of water absorption and germination showed little reduction in germination unless stored 10 days or longer.

The response of varieties to various storage conditions suggests the influence of such factors as the rate of water absorption, physiological activity of the embryos, and degree of dormancy of the embryos upon deterioration in storage. The contrast in these characteristics between the small-kerneled varieties and the large-kerneled Atlas and 2-rowed varieties has been demonstrated in numerous germination and malting experiments. The samples from Aberdeen, Idaho, that were harvested under dry conditions generally were more tolerant to poor storage conditions than those from Madison, Wisconsin, where environmental conditions seldom favor low moisture and physiologically inactive embryos, especially in combine-harvested grain.

The experiments indicate certain modifications in handling and storage of combine-harvested barley especially in the humid areas. Combine-harvested barley should be cleaned to remove green weed stems and seeds, insects, and other damp foreign material before the grain is put into storage bins or cars. The barley should be dried and aerated by frequent turning during the first 2 months of storage. Barley with high moisture content should be dried with high-velocity air at a temperature below 100°F.

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MINIMUM HUMIDITY REQUIREMENT FOR GERMINATION OF CONIDIA OF FUNGI ASSOCIATED WITH STORAGE OF GRAIN[1]

N. Armolik and J. G. Dickson[2]

SUMMARY

The spores of several mold fungi isolated from deteriorated barley differed considerably in their minimum humidity requirement for germination. Those of members of the *Aspergillus glaucus* group germinated on agar films under low moisture conditions or slightly above 70 per cent relative humidity (R. H.), conidia of *Penicillium* and *Aspergillus* species germinated at 79–81 per cent R. H., and conidia of *Fusarium moniliforme* germinated at 87.3 per cent R. H. The moisture content of the agar films ranged from 10 to 20 per cent. Spore germination at low relative humidities was a slow process and differed from that under higher moisture conditions. The relative rank of the several species for spore germination under low moisture supply was similar to that for spore germination at high salt or sugar concentration.

The determination of the minimum moisture requirements for spore germination of fungi associated with deteriorating grain has a direct bearing on the grain storage problem. Earlier investigations (1) showed that specific fungi accelerated the loss of germination in stored barley samples, especially immediately after harvest. Spores of some of this group of fungi were found commonly on grain from most areas. The rapid early development of the fungi and damage to the embryo appeared to be associated with the available moisture in the grain substrate, i.e. the surface layers of the kernels. Most barley samples stored immediately after combine harvesting developed sufficient moisture in the surface layers to stimulate spore germination and mycelial growth of some of these fungi even when the moisture content of the grain was below 14 per cent, a generally accepted safe moisture for grain storage.

The germination of spores of fungi causing deterioration of grains, foods, textiles, and other organic material has been studied under controlled humidities by several investigators (3, 4, 6, 7, 8, 10). An extensive review of the literature has been published in 2 recent papers (1, 9).

Galloway (6) considered that the molding of various materials was determined by the relative humidity of the storage atmosphere and moisture in the surface layer of the substrate rather than by the moisture content of the materials themselves. This was not confirmed by Block (3), who concluded that the water-absorbing properties of the substrate played an all-important role in determining the limiting relative humidity of the atmosphere under which mold growth occurred. The more hygroscopic the material, the lower the relative humidity at which the mold growth started.

Various methods have been used by different investigators for determining the minimum humidity requirement for spore germination, and the results frequently indicate disagreement. In most cases, the spore germination of mold fungi has been determined on gelatin sheeting (4, 6, 8, 9). Clayton (5) and Tomkins (11), working with pathogenic fungi, concluded that the influence of the material supporting the spores could be neglected. Quite different results were obtained by Groom and Panisset (7), who noted that the minimum relative humidity for spore germination of *Penicillium chrysogenum* on glass was 81 per cent whereas for mildewing of leather and other book materials it was 72.8 per cent. They suggested that the spores germinated at a lower relative humidity on book materials because of the presence of nutrients.

The minimum humidity required for spore germination in stored grain involves physical interactions difficult to analyze and control. Therefore, the relationship of relative humidity of the air and moisture content of exposed substrate to spore germination of specific storage fungi was determined under a simpler system. Malt-agar films have hygroscopic properties similar to that of the pericarp, lemma, and palea of the barley varieties compared. Also, the malt agar is similar in composition of fungus nutrients to the kernel tissues. Malt agar was used, therefore, as the substrate for determining water absorption and spore germination at several relative humidities.

MATERIALS AND METHODS.—Eight mold fungi, namely *Fusarium moniliforme* Sheldon, *Aspergillus flavus* Lk., *A. niger* v. Tiegh., *A. repens* (Cda.) d By., *A. amstelodami* (Mang.) Thom & Church, *A. ruber* (Bremer) Thom & Raper, *Penicillium cyclopium* Westling, and *P. chrysogenum* Thom, isolated from barley samples from various sources stored at high moisture, were used. Pure cultures of the molds were

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[2] Respectively, Graduate Assistant, Department of Plant Pathology; Professor of Plant Pathology and Agent, Department of Plant Pathology and Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Madison, Wisconsin.

TABLE 1.—*Average moisture content of agar film on cover glasses exposed to relative humidities of 70, 80, and 90 per cent, determined by increase in weight of 6 replicates*

R. H. in per cent	Moisture content of agar in per cent					
	1 Day	2 Days	4 Days	8 Days	14 Days	21 Days
70	10.2	10.1	10.1	10.4	10.1	10.8
80	13.6	14.0	13.9	14.2	14.0	15.0
90	23.9	24.4	24.4	24.8	24.7	25.1

grown on malt-salt agar slants. Cultures, after 2–3 weeks' growth on this medium, produced abundant dry spores that could be isolated in the dry state and used for inoculum.

The desired relative humidities were obtained by the use of sulfuric acid solutions of proper specific gravity, as given by Wilson (13). The spore germination on agar was carried out in large van Tiegham cells approximately 15 mm high and 20 mm in diameter. Two cells were sealed on a microscope slide with paraffin and filled to within 3 mm of the rim with sulfuric acid of proper concentration. One drop of melted malt-extract agar was placed on previously flamed cover slips and dried 3 days in sterile Petri dishes. Platinum wire was used for seeding the agar with spores and for spreading the spores uniformly. The cover slips carrying the spores were then inverted and sealed with vaseline over the top of the van Tiegham cells. The cultures were held in a room where the average temperature was 25° ± 2°C. At relative humidities between 70 and 85 per cent, this change in temperature was considered within the range of experimental error. The cultures were incubated and at regular intervals were examined under the microscope. The moisture absorbed by the malt-agar film was calculated from data obtained by weighing a replicated set of tared cover slips with agar exposed to 3 different relative humidities (Table 1). The 0.5 ml of agar on each cover slip was dried to constant weight over calcium chloride and weights determined. Six replicates of cover slips with agar were placed in each of 3 closed containers with atmospheres adjusted to 70, 80, and 90 per cent relative humidity and weighed at time intervals. The moisture content, expressed as percentage increase in weight of agar at equilibrium with the moisture in the air of the 3 containers, showed a straight line relationship over the range of relative humidity tested. The moisture contents of the agar films in the spore-germination tests were calculated from an interpolation of the log-log graph of these data.

The capacity of spores to germinate at high concentrations of salt was determined in Petri dishes on malt-salt agar containing different concentrations by weight of NaCl ranging from 10 to 40 per cent at intervals of 2 per cent. During an incubation period of 25 days at 25°C, spore germination, mycelial growth, and sporulation of the fungi were determined.

Tests on spore germination in solutions of sucrose were carried out in 150-ml Erlenmeyer flasks containing 30 ml of solution. The solutions contained different concentrations of sucrose by weight ranging from 40 to 68 per cent at intervals of 2 per cent. The cultures were incubated at 20°C for 25 days. The osmotic value of the sucrose solution and the corresponding relative humidity equivalent was determined from the data of Walter (12; p. 159, 161). As these tables did not give the osmotic values for high concentration of sucrose solutions, the reported values were found by graphical interpolation.

EXPERIMENTAL RESULTS.—*The relation of relative humidity to spore germination, fungus growth, and sporulation on agar films.*—The mold species studied varied considerably in their minimum moisture requirements for spore germination (Table 2). Conidia of *Fusarium moniliforme* germinated on an agar film at 87.3 per cent R. H. or 20.4 per cent moisture, the highest moisture requirement of the fungi studied. The percentage of germination was low at this humidity, and the germ tubes were abnormally thickened. Spores of *Aspergillus flavus*, *A. niger*, *Penicillium cyclopium*, and *P. chrysogenum* were similar in minimum moisture requirements (79-81 per cent R. H.). Spores of members of the *A. glaucus* group showed

TABLE 2.—*Minimum relative humidities, maximum salt concentrations (in malt agar), and maximum sucrose concentrations (in liquid media) at which spores of various fungi germinated*

Fungus	On malt-agar films over sulfuric acid in sealed dishes		Maximum NaCl concentration in malt agar at which germination occurred	Data on most concentrated sucrose solutions in which germination occurred		
	Minimum R. H. at which germination occurred	Calculated moisture of agar film		Sucrose content by weight	Osmotic pressure of solution	Calculated R. H. equivalent
	%	%	%	%	atm.	%
Fusarium moniliforme	87.3	20.4	14	60	154	89.0
Penicillium cyclopium	81.0	15.7	20	64	172	87.9
P. chrysogenum	79.0	14.5	22	67[a]	217[a]	85
Aspergillus flavus	80.2	15.1	20	64	172	87.9
A. niger	79.0	14.5	22	67	217	85
A. repens	71.5	11.2	28	67	217	85
A. ruber	71.5	11.2	26	67	217	85
A. amstelodami	70.3	10.9	30	67	217	85

[a] Germination occurred at this concentration, which is maximum solubility of sucrose.

a greater capacity to germinate under low moisture conditions than did those of other fungi tested. Spores of *A. amstelodami* germinated at 70.3 per cent R. H. or 10.9 per cent moisture during the 4 months' incubation period, whereas those of *A. repens* and *A. ruber* required 71.5 per cent R. H. or 11.2 per cent moisture. Three of the fungi, *A. amstelodami*, *A. repens*, and *A. ruber*, developed on agar films containing less than 12 per cent moisture, a value below the average moisture content of stored grain. All fungi studied developed at moistures below that of most grain harvested by combine and stored immediately after harvest.

The type of spore germination under restricted moisture conditions of all *Aspergillus* and *Penicillium* species studied was different from that at the higher humidities. The spores, before the initiation of hyphae, swelled to several times the diameter of the original spore. The hyphae were at first thickened with frequent cross walls, but they became normal in appearance in later growth. Usually the growing tips of hyphae were swollen.

The relation of salt concentration of agar to spore germination and growth.—Spores of the several fungi studied differed also in their capacity to germinate on agar containing high concentrations of NaCl. In general, the species showed the same ranking for spore germination at different salt concentrations as in the previous experiments over a range of relative humidity values. Mycelial growth and sporulation of the several fungi occurred at salt concentrations slightly lower (2–4 per cent) than that inhibiting spore germination.

The relation of sucrose concentration to spore germination and growth.—Spore germination in increasing sucrose concentrations was studied primarily to determine the relative osmotic pressure values necessary to inhibit spore germination of the several fungi. Snow (10), Walter (12), and others (9) have studied the physical properties and biological reactions conditioned by sucrose concentrations. With these data available, comparisons were made to check further the results obtained on minimum relative humidity and maximum salt concentration required to prevent spore germination. The relative relationship between osmotic pressure of the solution and the equivalent relative humidity value has been studied by Walter (12). On the basis of Walter's equations, the highest concentration of sucrose that permitted spore germination of *F. moniliforme*, 60 per cent, was calculated as equivalent to a relative humidity of 89 per cent, or 1.7 per cent higher than the relative humidity required for the spores on the agar film (Table 2). The spore germination of the other fungi studied occurred at such high sucrose concentrations that accurate comparisons were impossible. Spores of *P. cyclopium* and of *A. flavus* germinated at a maximum sucrose concentration of 64 per cent. Spores of the other fungi germinated and mycelium developed in saturated solutions of sucrose or at calculated osmotic pressures of 217 atmospheres or above.

DISCUSSION.—The data on moisture requirements for spore germination and fungus development indicated that this group of grain storage fungi developed on substrates at moisture contents between 10 and 20 per cent. The members of the *Aspergillus glaucus* group developed under lower humidity conditions than did the others, and they were usually the first fungi to invade cereal grains in storage. *A. amstelodami*, of the 3 members in this group studied, showed the greatest ability to grow under low moisture conditions. These results were somewhat different from those obtained by Snow (10), who placed *A. ruber* as the lowest of this group in ability to germinate at low moisture levels. Possibly strain differences among the mold fungi partly explain the differences in results obtained.

It is unlikely that the actual molding of grain occurs at the minimum humidity required for spore germination (8, 10). The abnormalities in spore germination and germ tube development at the minimum humidities suggested difficulties in fungus establishment in the tissues at these minimum moisture levels; however, germ tube formation was usually quite normal at R. H. values 1 to 2 per cent higher than the minimum for germination. *A. flavus* and *A. niger* produced conidiophores and conidia at moistures slightly above the minimum R. H., whereas members of the *A. glaucus* group were able to reproduce when the relative humidity exceeded 75 per cent.

The spore germination in humid air was dependent upon the hygroscopicity of the substrate, as shown by Block (3). Agar and malt-extract agar were sufficiently hygroscopic to allow spores placed on them to germinate at a lower relative humidity than did spores on some other materials. At these minimum R. H. values, no spores germinated on areas of dry glass cover slips not covered by the agar drop. This indicated that the spores were incapable of absorbing moisture for spore germination directly from the atmosphere but derived it from the substrate. Spore germination on dry glass occurred, however, at higher relative humidities. A few spores of *A. amstelodami* germinated on dry glass at 85 per cent R. H. The surface layers of the kernels of grain are similar to the agar films in hygroscopic properties, but they were not used as a substrate since it was impossible to obtain kernels free from microflora.

The establishment of equilibrium between the humid air and the agar under sterile conditions occurred rapidly, but there was a slight tendency for the agar to absorb more moisture as the length of exposure was increased. This may partly explain the slow germination at the minimum humidities.

The use of concentrated salt or sugar solutions for comparison with the minimum moisture requirement and maximum osmotic pressure for spore germination was studied earlier by Heintzeler (8). The various salts used gave different results. $CaCl_2$ appeared to be more toxic to spore germination than was KCl or NaCl, and use of sugar solutions gave higher minimum R. H. values than did direct germination in

humid air. The use of sugar solutions for determining the minimum moisture requirements for spores of many of the mold fungi was limited, because many germinated in saturated sugar solutions.

The data obtained on spore germination and fungus development indicated that some of these fungi are equipped physiologically to grow in stored grain at moisture contents commonly considered safe for grain storage. The moisture content of freshly combined barley, especially in the humid areas, was found to be above the minimum required for the rapid growth of all of this group of fungi. Other investigations (2) have shown that the development of these fungi in stored barley contributes to the reduction in germinability of the grain during a relatively short period of storage. Longer periods of storage at high moistures result in moldy grain.

These and other investigations on the relation of fungi to grain deterioration in storage indicate the need for artificial drying of combine-harvested grain to assure good quality and high germinability. The critical period in relation to the influence of fungi on germinability appears to be in the first few days following harvesting or while the barley is in farmers' bins or in transit in railroad cars.

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REPORT OF THE 1955-1956 MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The thirteenth annual meeting of the Potomac Division of the American Phytopathological Society was held March 1 and 2, 1956, in the auditorium of the Plant Industry Station, Beltsville, Maryland. Approximately 250 persons attended.

Thirty-one scientific papers were presented. The annual banquet was held in the St. Andrews Episcopal Parish House, College Park, Maryland. Following the banquet, Mr. Fred Packard, National Parks Association, spoke on "Exploration of our National Parks and Monuments." During the second day, a symposium on "Market Pathology" was held with Dr. H. T. Cook as chairman. The topics and speakers were 1) History and Development of Market Pathology, H. T. Cook; 2) Control of Market Diseases by Means Other Than Fungicides, L. P. McColloch; 3) Control of Postharvest Diseases by Fungicides, W. L. Smith, Jr.; 4) Market Diseases in a Large City Market, B. A. Friedman; and 5) Transportation Problem in Relation to Postharvest Market Diseases, W. H. Redit.

The following officers were elected to serve during the next year: *President*, J. G. LEACH; *Vice-President*, KERMIT W. KREITLOW; and *Councilor*, W. D. MCCLELLAN.

L. O. WEAVER, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Control of downy mildew of muskmelons with tank mixtures of a fixed copper and a dithiocarbamate. BEECHER, F. S. In the 1955 tests at Beltsville, Maryland, downy mildew (caused by *Pseudoperonospora cubensis*) of muskmelons was controlled successfully with several mixtures of tribasic copper sulfate and a dithiocarbamate, Dithane Z-78 (65 per cent zineb). There were approximately 48 vines per plot, replicated 4 times. Weekly spray applications were made with a small power sprayer that had 2 nozzles on a single hand rod and was operated at about 200 lb. pressure. A sticker-spreader, Triton B-1956 (phthalic glycerol alkyl resins), was included in all sprays. All fungicides were diluted separately before they were added to the tank. When 1, 2, or 3 lb. of the copper fungicide was used with 1 lb. of zineb per 100 gal. of spray, control was about equal to that with 2 lb. of zineb alone. In all combinations, 2 lb. of zineb proved better than 1 lb.; however, combinations of 1, 2, or 3 lb. of tribasic copper sulfate with 2 lb. of zineb per 100 gal. gave better mildew control than did either fungicide applied separately or in an alternating schedule. Varying the amount of the copper fungicide from 1 to 3 lb. in the mixture with 2 lb. of zineb resulted in no significant differences in control. No foliage injury due to the fungicides was observed.

Detection and transmission of hydrangea ringspot virus. BRIERLEY, PHILIP, AND PAUL LORENTZ. Hydrangea ringspot virus is transmissible by manual methods and by the cutting knife but apparently not through seed. It produces distinctive local lesions on *Gomphrena globosa* and on *Chenopodium amaranticolor* after 5–6 days. It is also infectious to snapdragon, sweet william, *Amaranthus retroflexus*, and sugar beet but not to tobacco, China aster, bean, or guar. When extracted from aging leaves in January, the virus withstood heating for 10 minutes at 70°C, dilution to 10^{-3}, and aging for 12 days at 20°. It was found in all 9 commercial varieties of *Hydrangea macrophylla* tested. Two newer varieties, Sainte Therese and Todi, were found only partially infected. Some florists believe that hydrangea varieties "run out." Hydrangea ringspot virus is the only known virus of this species sufficiently prevalent to have a role in decline of varieties. Tomato ringspot virus, which can also be detected in hydrangea by sap inoculation to Gomphrena, occurs infrequently in hydrangea, but it is more damaging to hydrangea than is hydrangea ringspot virus. No evidence was found that tomato ringspot virus is carried either on the cutting knife or through seed.

Weather fleck leaf lesions on tobacco. BURK, L .G., AND H. E. HEGGESTAD. During the 1955 season, studies of weather fleck, a leaf-spot disease, were made following severe outbreaks that occurred in tobacco field plantings at the Plant Industry Station, Beltsville, Maryland. Accelerated vegetative activity in the presence of excessive moisture and prolonged periods of high humidity favored the formation of leaf spots. Following a predisposing environmental period, closely aggregated, irregular, brownish-to-tan flecks appeared. Most lesions were 2 mm or less in length and often coalesced, causing large portions of leaves, or entire leaves, to collapse. After the first appearance of the leaf spot, about mid-July, there were successive periods when new fleck lesions developed on younger leaves higher on the stalk and on healthy tissue of previously affected lower leaves. Repeated failures to isolate specific pathogens from new lesions or to detect them in microscopic preparations suggest that weather fleck results from physiologic factors. In a replicated test with 4 varieties and their F_1 hybrids, fleck was severe on 2 Burley varieties, Burley 11-A and Burley 2, intermediate on flue-cured Virginia Gold, and very mild on Maryland Medium. Maryland Medium hybrids had comparatively low fleck indices, indicating a dominant expression of the Maryland parent.

Resistance of oat varieties to two species of root-knot nematodes. CRITTENDEN, H. W. Data obtained in Delaware during a search for grain varieties resistant to *Meloidogyne hapla* and *M. incognita* var. *acrita* showed that all varieties of barley and wheat tested were resistant to the former species and susceptible to the latter. With oat varieties, however, the results indicated that all varieties tested were resistant to *M. hapla* but that a difference existed in the reaction of varieties to *M. incognita* var. *acrita*. The oat varieties susceptible to the latter species were as follows: Forkedeer, Lee, Letoria, Stanton, Traveler, Fulwin, Arkwin, Leconte, and Dubois. Resistant oat varieties were Arlington, Atlantic, Mustang, Lemont Cross, Coy, Frazier, De Soto, Victorgrain, and Victoria. Since both nematode species are present in Delaware, it is fortunate that adapted varieties resistant to both species are available. These varieties have a place in crop rotations for control of root-knot nematodes by their use as a winter cover crop as well as a crop for harvest of grain.

Carbohydrate fermentation by Xanthomonas vesicatoria. CROSSAN, D. F., AND L. R. KRUPKA. Using standard colorimetric procedures, some workers have demonstrated acid production from sugars by *X. vesicatoria*, whereas others have been unable to do so. In the work reported herein, in which the indicators brom cresol purple, brom thymol blue, and phenol red in a 1 per cent sugar-mineral medium were

used, it was possible to show growth but not acid production. As it is known that alkalinity increases following growth of the organism in media containing a protein source, subsequent tests were made in a medium containing 1 per cent ammonium phosphate, 0.2 per cent potassium chloride, 0.2 per cent magnesium sulfate, and 1 per cent of the carbohydrate under test. No indicators were used. Each sugar was replicated 4 times in Erlenmeyer flasks containing 20 ml of solution. Flasks were seeded and incubated under constant agitation. Nonseeded flasks served as checks on the initial pH of the solution. After 96 hours' incubation, the pH of each solution was determined with a Beckman pH meter. Fermentation was indicated by a consistent drop in pH below that of the checks. Fermentation could be demonstrated for dextrin, fructose, galactose, glucose, sucrose, and trehalose but not for arabinose, dulcitol, glycerol, inulin, lactose, maltose, mannitol, raffinose, rhamnose, salicin, and xylose.

Further results on the vertical advance of two "house decay" fungi in artificially infested timbers. DILLER, J. D., AND S. W. HUANG. Two years ago, at the 11th Annual Meeting of the Potomac Division of the American Phytopathological Society, the authors reported an experiment in progress in which wood of 3 species was infested with pure cultures of the 2 "house decay" fungi, *Merulius lacrymans* Jacq. ex Fr. and *Poria incrassata* (Berk. & Curt.) Burt., in an abandoned root cellar at Beltsville, Maryland. Upended boards (2 × 4 in.), representing the 3 wood species, were left undisturbed under the fluctuating root-cellar conditions. After 4 years, *M. lacrymans* has now advanced an average of 5.1 ft. in all test pieces. During the same period, *P. incrassata* advanced only 4.5 ft. Both fungi advanced farthest in the southern pine timbers, averaging 5.5 and 5.1 ft., respectively, for *M. lacrymans* and *P. incrassata*. *M. lacrymans* advanced 4.9 and 4.7 ft. in oak and Douglas fir, respectively; and *P. incrassata* advanced 4.5 and 3.9 ft. in Douglas fir and oak, respectively.

Further results on the relative durability of 40 wood species to "house decay" fungi. DILLER, J. D., AND E. J. KOCH. Two years ago, at the 11th Annual Meeting of the Potomac Division of the American Phytopathological Society, the author described a method for successfully growing *Poria incrassata* (Berk. & Curt.) Burt. and *Merulius lacrymans* Jacq. ex Fr. under mounds of forest leaves in a wooded area near Beltsville, Maryland. Wood of 40 species (7268 test blocks) were subjected to the decay action of each fungus at exposure intervals of ½, 1, 1½, and 2 years. The fungi remained healthy and active throughout the 2 years. *M. lacrymans*, for the most part, exhibited greater wood-destroying ability than did *P. incrassata;* in 1 case, the former species produced a normal fruiting surface. The 3 most durable woods were Osage orange, Japanese chestnut, and American smoketree. The 3 least durable were eastern and western hemlock and Douglas fir.

New concepts of the Verticillium wilt disease of maple. DOCHINGER, L. S. Field surveys for Verticillium wilt of maple were conducted in 27 New Jersey localities during 1954-1955. *Verticillium* sp. was isolated or symptoms of the disease were present in 3.4 per cent of the trees. The disease was most prevalent in silver maple (*Acer saccharinum* L.), followed by Norway maple (*A. platanoides* L.), red maple (*A. rubrum* L.), and sugar maple (*A. saccharium* Marsh), with 8.0, 2.8, 1.4, and 1.0 per cent, respectively. Potted Norway maple trees were supplied at 2-day intervals with complete nutrient solutions that varied only in nitrogen content (14, 70, and 154 ppm). After 160 days of treatment and 85 days following trunk inoculations with *Verticillium* sp., the dissected trees showed not only an increase in amount of callus around the wounded areas as the nitrogen supply was raised but also an increase in the upward discoloration by Verticillium. Sapwood wounds were made on field-grown Norway maple trees with an ax, saw or increment borer previously used on Verticillium-infected trees. Two months later, cultures made from symptomatic discolorations that developed in sapwood under the wounds yielded *Verticillium* sp. in approximately 10 per cent of the cases.

Progress in breeding for resistance to clubroot of broccoli and cauliflower. GALLEGLY, M. E. Plants resembling broccoli and cauliflower and possessing resistance to clubroot (incited by *Plasmodiophora brassicae*) have been selected from crosses of a resistant cabbage with commercial broccoli and cauliflower varieties. The resistant cabbage breeding line, 83-595s, was obtained from J. C. Walker, University of Wisconsin. Segregation data indicate that resistance is controlled by multiple genes. Backcrosses are being made to obtain commercially acceptable clubroot-resistant broccoli and cauliflower varieties.

Resistance of Nicotiana knightiana and other tobacco species to root-knot nematodes. HEGGESTAD, H. E., AND J. J. GROSSO. In 1955, promising new sources of resistance to root-knot nematodes were found in *Nicotiana* species. Plants of 48 *Nicotiana* species were inoculated by means of chopped galled roots containing predominantly *Meloidogyne incognita* var. *acrita*. In both greenhouse and field experiments, inoculated plants of *N. knightiana* Goodsp. developed more extensive fibrous root systems than did other species. Plants were either free of galls or had relatively few small galls. Microscopic examination of galls revealed that those female nematodes found were retarded in development, a further indication that this species was highly resistant. Thirty-two of the 48 *Nicotiana* species tested were quite susceptible to root-knot. *N. occidentalis* was one of the most susceptible species. *N. repanda* was the only species observed free of galls. *N. repanda* has been reported previously as root-knot resistant, but successful crosses with *N. tabacum* have not been made. A vigorous *N. tabacum* × *N. knightiana* hybrid has been made by R. A. Steinberg. Cuttings will be used to determine the resistance of the F_1 hybrid to root-knot and other parasitic nematodes. Other *Nicotiana* species showing good root-knot resistance but requiring further testing include *N. arentsii*, *N. otophora*, and *N. paniculata*.

Control of Botryosphaeria rot and sooty blotch on apples. HEUBERGER, J. W., R. R. ROMANKO, AND M. Q. SAYED. During 1952, the rot incited by *Botryosphaeria ribis* caused a 75 per cent loss of fruit in a block of the Rome variety of apple at Bridgeville, Delaware. Tests in which captan and zineb were used were conducted in this block for the period 1953–1955, inclusive. In 1953, the materials were used in all-season schedules, whereas in 1954 and 1955 they were used only in the cover spray period (fourth through eighth cover). In all 3 years, both diseases were present. Data on percentage fruit infection by *Botryosphaeria ribis* in 1953, 1954, and 1955, respectively, were as follows: unsprayed—11, 37, 58; captan—0, 6, 15; zineb—1, 9, 9; captan + zineb (half strength of each), 1954 and 1955 only—7, 12; captan and zineb (alternating), 1955 only—13. Percentage sooty blotch fruit infection was as follows: unsprayed—91, 27, 40; captan—64, 25, 27; zineb—1, 11, 12; captan + zineb (half strength of each), 1954 and 1955 only—7, 11; captan and zineb (alternating), 1955 only—9. Data on sooty blotch control on Red Delicious, Golden Delicious, Stayman, and Blaxtayman varieties (Newark, Delaware, 1955) confirmed the above control data in showing that captan was ineffective, that zineb was highly effective, and that captan + zineb was as effective as zineb alone.

Indicator plants for cork virus transmission dependent on photoperiod control. HILDEBRAND, E. M., AND H. A. BORTHWICK. Plants of Scarlett O'Hara morning glory, probably an Ipomoea hybrid, and several other related species or forms were found to be satisfactory indicators for indexing internal cork virosis of sweetpotato (*Ipomoea batatas*). Although Scarlett O'Hara was best, others obtained from commercial sources under the names *Ipomoea hederacea*, Tennessee Purple Ipomoea, *Pharbitis hederacea*, and *P. diversifolia* also proved useful. After mid-September, because of the onset of early flowering, plants of all these species suddenly lose their effectiveness as indicators. Their indexing value depends upon their continued production of vigorously growing shoots. This type of growth ceases with

the onset of conditions favorable to early flowering. The abrupt transition from vegetative to reproductive development in September indicates that the plants are controlled by daily duration of photoperiod, a fact previously established by other workers for some of these varieties, forms, or species. Accordingly, plants were subjected to 3 hours of light during the middle of the night, beginning at emergence. Under these conditions, there was vigorous vegetative growth without flower formation. Briefer periods of light probably would have been adequate, but they were not tested.

Aphid transmission of sweetpotato cork virus in the greenhouse. HILDEBRAND, E. M., AND F. F. SMITH. Internal cork virus has been transmitted by *Myzus persicae* (Sulz.) from sweetpotato to sweetpotato, from *Pharbitis diversifolia* to Scarlett O'Hara morning glory, from Scarlett O'Hara to Scarlett O'Hara, and from sweetpotato to Scarlett O'Hara. *Macrosiphum solanifolii* also transmitted the virus. Aphids from cabbage were starved for 1–2 hours, fed on diseased sweetpotato foliage for 10 minutes, transferred to healthy plants for 2 hours, and then destroyed. The inoculated plants were grown to maturity. At harvest, root lesions were rare on these plants and on graft-inoculated plants in a parallel series. After 6 months storage, roots of less than 20 per cent of the inoculated plants had lesions, whereas about 75 per cent of the sprouts from the symptomatic and symptomless roots produced typical chlorotic spot symptoms. Hence, the sprout symptoms appear to be the more reliable criterion of transmission. In Scarlett O'Hara plants, the incubation period was 6–10 days following transmission of the virus, either mechanically or by aphids, from sweetpotato, *P. diversifolia*, or Scarlett O'Hara.

Efficacy of soil treatments in tobacco black shank control. LAUTZ, WILLIAM. Allyl bromide, chlorobromopropene, copper sulfate, Dithane D-14 (19 per cent disodium ethylenebis[dithiocarbamate]), Dowicide A (97 per cent sodium *o*-phenylphenoxide), formaldehyde, methyl bromide, sodium azide, urea, and N869 (*N*-methyl dithiocarbamate dihydrate) were tested to determine effectiveness as soil treatments in controlling black shank (caused by *Phytophthora parasitica* var. *nicotianae*) in 6 experiments conducted over a 5-year period. One 6-in. tobacco plant of the 402 variety was transplanted to each of 5 or 10 glazed crocks of infested soil previously treated with 1 of the above chemicals. Plants also were grown in untreated infested soil and in disease-free soil. Disease development on roots and tops was noted. Methyl bromide (1 lb./100 sq. ft.), which was tested only once, controlled black shank. N869, 1/40; Dithane D-14, 1/50; and formaldehyde, 1/25, were highly effective as drenches against black shank, and treatments resulted in no phytotoxicity. They gave complete control in at least 1 test and fair to excellent control in other tests, depending upon conditions of treatment. Less concentrated drenches of formaldehyde and of Dithane D-14 were found to be inferior in disease control. Erratic results were secured with the other chemicals.

Cytological changes in Macrosteles fascifrons fed on plants infected with aster yellows virus. LITTAU, VIRGINIA C., AND KARL MARAMOROSCH. Nonviruliferous aster leafhoppers, *Macrosteles fascifrons* Stäl, were bred in a stock colony maintained on rye plants. Viruliferous insects were obtained by feeding stock insects for 1–2 weeks on yellows-diseased asters and subsequently transferring them to a succession of fresh healthy China asters. For a cytological study, 42 viruliferous adult insects and 38 nonviruliferous insects of comparable age were fixed in Carnoy's fluid and stained with safranin, azure B (pH 4), and a triple stain for deoxyribonucleic acid, polysaccharide, and protein. In nonviruliferous insects, the nuclei of fat-body cells tended to be round and to have smooth contours; only a few were stellate and these were observed in only ⅓ of the leafhoppers. The cytoplasm, generally homogenous with a large number of vacuoles of varying size, in some cases stained so heavily that nuclei were barely discernible. In viruliferous insects, almost all nuclei of the fat-body cells were stellate and the cytoplasm was reticulate and stained less intensely; many cells seemed abnormal and appeared broken. Although viruliferous leafhoppers live as long and breed as freely as nonviruliferous individuals, the cytological differences suggest that aster yellows virus causes a disease in its insect vector.

A rhizopus rot of gladiolus corms. MCCLELLAN, W. D. A light-brown soft rot of gladiolus corms caused by *Rhizopus* sp. was encountered during studies on the treatment of freshly harvested gladiolus corms. Little or no odor accompanies this rot. High temperature, high humidity, and wounding of freshly harvested corms favor its development. The percentages of rot in Spotlight corms that were wounded, inoculated, and placed in a saturated atmosphere for 16 days at 95°, 85°, 75°, and 65°F, were 100, 66.2, 0.0, and 0.0, respectively. Only 7.3 per cent of the wounded corms rotted when kept for the same length of time at low humidity at 95°F, whereas 82.5 per cent of unwounded corms rotted when inoculated and placed at 95°F and high humidity. Corms produced from cormels were slightly more susceptible than those produced from large corms. A 5-day curing period at 85°F prior to inoculation did not prevent rot. Picardy and October Sunshine were most susceptible of the varieties tested, Chamouny and Spic and Span were least susceptible, and Spotlight was intermediate in susceptibility. Soft rot bacteria frequently follow the Rhizopus rot, but if not the corms mummify and become very hard. It is expected that Rhizopus rot will be of relatively little commercial importance.

A new method of infecting Pinto bean stems uniformly with southern bean mosaic virus. MITCHELL, J. W., W. H. PRESTON, JR., AND J. M. BEAL. Small areas on the upper portions of Pinto bean hypocotyls were inoculated with southern bean mosaic virus by 1) applying drops of inoculum separately to the hypocotyls, 2) introducing a small amount of Carborundum into each drop, 3) rubbing the area under each drop lightly with the flat, slightly roughened end of a glass rod, and 4) washing off inoculum and Carborundum with tap water immediately after inoculation. This technique injured only the epidermal and perhaps some subepidermal cells. Within 2 days, a slightly sunken necrotic area, involving epidermal and cortical cells, developed at each inoculated region. Later, cells of the endodermis, pericycle, and phloem became necrotic. A limited region containing these last 3 tissues soon became necrotic and extended up and down the entire hypocotyl. This necrotic strip and tissues in and adjacent to the inoculated area contained detectable amounts of the virus. The technique described resulted in limited systemic virus infection in an otherwise typical "local-lesion-type" plant.

Effects of antibiotics on Colletotrichum sp.—the cause of anthracnose of tobacco. MORGAN, O. D. In tests with 6 antibiotics—aureomycin, terramycin, neomycin, Agrimycin, streptomycin, and Ilotycin—on the control of anthracnose on tobacco, more lesions were produced on leaves treated with 200 ppm of an antibiotic than on untreated checks. This was closely correlated with heavier sporulation on potato-dextrose agar treated with 200 ppm than on untreated checks. Sporulation occurred earlier on treated than on untreated plates. At higher concentrations of antibiotics, less sporulation occurred. The rate of growth of the fungus on 5 antibiotic-treated media decreased rapidly as the antibiotic concentration was increased up to 400 ppm. Above 400 ppm, growth rate leveled off, and with 4 of the antibiotics, it increased at 800 ppm. On aureomycin-treated media, the growth rate decreased steadily up to 800 ppm, then leveled off. Fewer lesions occurred on plants treated at the higher concentrations than on untreated checks. Concentrations used on the plants did not exceed 400 ppm because of injury above this concentration. Aureomycin gave the best control of the fungus on plants and in the medium. Streptomycin sulfate gave least control on plants and in the medium.

Resistance in the F_1 progeny of the cross var. Maryland Catterton × Nicotiana sylvestris and var. Maryland Robinson × N. sylvestris to tobacco anthracnose. MORGAN, O. D. *Nicotiana sylvestris*, which has a moderate degree of resist-

ance to tobacco anthracnose, was used as the staminate parent in crosses on 2 Maryland named varieties, Catterton and Robinson. The F_1 progeny and parents were inoculated at the 6-leaf stage to determine if the resistance was carried over in the cross. Most lesions on *N. sylvestris* were very small (1 mm or less in diameter) and brownish. Occasional larger lesions occurred up to 3 mm in diameter. Lesions on the Maryland varieties were generally 3–4 mm. Lesions on the veins and petiole of *N. sylvestris* were about 1 mm wide and 3–4 mm long. Lesions on the tobacco varieties were large, up to 10 mm long and half as wide. The criterion used for eliminating susceptible plants in the F_1 progeny was the presence of numerous large lesions. About ½ of the F_1 plants of both crosses had lesions similar to those on *N. sylvestris*. Lesions on the F_1 plants were slightly more numerous than those on the resistant parent. All the plants of *N. sylvestris*, of the F_1 progeny, and of Maryland Catterton survived inoculation. All the Marvland Robinson plants died. There appears to be varietal differences in susceptibility. This difference was not carried over in the F_1 plants.

Longevity of cleistothecia of Erysiphe graminis f. sp. hordei. Moseman, J. G., and H. R. Powers, Jr. Cleistothecia of *Erysiphe graminis* f. sp. *hordei* Em. Marchal from plants grown at 5 locations in North America were used to study techniques for obtaining cultures from ascospores. Similar responses to various combinations of moisture conditions and temperatures were observed in the cleistothecia from plants grown at the 5 locations. Cardinal temperatures for the discharge of ascospores were found to be 8°, 16°–20°, and 24°C, which were the same as those for the germination of ascospores, except for the maximum which was 28°. Infection with ascospores was obtained most readily when the cleistothecia were kept continuously moist at a constant temperature of 18°. Cleistothecia in mycelial mats on leaves were found to be viable following storage for over 13 years in dry envelopes at either room temperature or at 10°C in a refrigerator.

Effects of absorbed and translocated F-17 culture filtrate antibiotic factors on the bean rust organism. Preston, W. H., Jr., E. J. Daly, B. C. Smale, and J. W. Mitchell. A crude culture filtrate from *Streptomyces cinnamoneus* F-17 (supplied by Northern Utilization Research Branch, Agricultural Research Service) contained antibiotics that controlled the bean rust pathogen, *Uromyces phaseoli* var. *typica*, under greenhouse conditions even when diluted to 1/8 of the original concentration. When the diluted filtrate was applied to the upper surfaces of bean leaves and the lower surfaces were inoculated, antibiotics in the filtrate were absorbed and moved within the leaves in sufficient amounts to prevent symptom formation. The antibiotics were absorbed and translocated upward to inoculated primary leaves, where symptom expression was prevented, when the filtrate was applied to stems. The undiluted filtrate applied to the leaves 4 days after inoculation prevented spore formation. Applied to stems at the time of inoculation, the antibiotics reduced spore production and also the infectivity of those spores that matured.

Antibiotics against plant disease. I. Laboratory and greenhouse survey. Pridham, T. G., L. A. Lindenfelser, O. L. Shotwell, F. H. Stodola, R. G. Benedict, C. Foley, R. W. Jackson, W. J. Zaumeyer, W. H. Preston, Jr., and J. W. Mitchell. About 500 pure cultures of *Streptomyces* were subjected to primary screening by 3 methods against a minimum of 12 phytopathogenic bacteria and fungi. Based on their antimicrobial spectra, some 200 strains were selected as warranting further study and were grown on a variety of media in shaken-flask fermentations. The culture filtrates were tested for antibiotic activity by paper-disc assay against 9 phytopathogenic bacteria and fungi. Fifty-two of the 200 strains showed promise. Culture filtrates from 8 strains and 2 substances isolated from the mycelia of different strains have been tested thus far in the greenhouse against 2–10 plant diseases. Nine of the 10 products showed broad antifungal spectra in laboratory tests and activity against at least 1 disease in greenhouse tests. Phytotoxic effects were observed with some of the test materials.

Antibiotics against plant disease. II. Effective agents produced by a new strain of Streptomyces cinnamoneus. Pridham, T. G., O. L. Shotwell, F. H. Stodola, L. A. Lindenfelser, R. G. Benedict, and R. W. Jackson. A strain of *Streptomyces cinnamoneus* has been found to produce a mixture of antibiotics that is antagonistic to several phytopathogenic bacteria and fungi in the laboratory. This Streptomycete is characterized by the whorled morphology and cinnamon-pink color of its sporulating aerial mycelium. We consider it to be a physiological form of *S. cinnamoneus*. The mixture of antibiotic agents is produced when the organism is grown in a medium of tap water, soybean meal, and glucose. Tests conducted in the greenhouse show that culture filtrates are effective against rust, anthracnose, downy mildew, and powdery mildew of beans; against stem rust of wheat; and against powdery mildew of bluegrass. Paper chromatographic studies reveal that 3 antibiotics are present in the mixture. Assays in the laboratory and bean rust assays performed in the greenhouse on numerous chemical fractions indicate that no one single antibiotic is responsible for the activity against bean rust. Potency is retained in the dried filtrate.

Host range of Trichodorus sp. and its host-parasite relationships on tomato. Rohde, R. A., and W. R. Jenkins. *Trichodorus sp.*, the stubby-root nematode, occurred in 45 of about 1000 soil samples collected in Maryland during 1955. This nematode was found only in light sandy soils and most commonly in the vegetable- and tobacco-growing regions. In 70 per cent of the samples where specimens of *Trichodorus* sp. were found, they were associated with corn, soybean, tomato, or tobacco. In sterilized soil in the greenhouse, populations of *Trichodorus* sp. were supported for 60 days or more by 51 different plant species representing 15 plant families. In pots not containing living plants, no specimens of *Trichodorus sp.* were found after this time. Only 4 plant species, *Asparagus officinalis, Crotalaria spectabilis, Datura stramonium,* and *Euphorbia pulcherrima,* did not support populations. Control by rotation would, therefore, be impractical in most cases. In greenhouse-grown Chesapeake variety tomatoes, symptoms produced after inoculation with *Trichodorus* sp. consisted of general stunting plus shortening and malformation of lateral roots near the base of the stem. Histological studies and observations of feeding indicate that damage is caused by decreased cell multiplication in root tips rather than by mechanical destruction of cells.

Fusarium wilt of carnations retarded by fungus-eating nematodes, Ditylenchus spp. Schindler, A. F., and Robert N. Stewart. Previous experiments have indicated the possibility that certain species of *Ditylenchus* inhibit bacterial wilt of carnations. Since these nematodes are known to be mycophagous, experiments were initiated to determine whether they might inhibit Fusarium wilt, a fungus disease of carnations. A total of 160 rooted carnation cuttings was used in 4 replications of a 2×2 factorial arrangement (4 treatments) in an initial experiment with both *Fusarium oxysporum* f. *dianthi* and *Ditylenchus* spp. In treatments in which nematodes were included, approximately 3200 specimens were pipetted onto the roots of each plant at the time of potting. The fungus was added to the soil immediately after potting. After 17 days, no plants in either the check or the treatment with nematodes alone showed wilt symptoms. Where the fungus was added, an average wilt rating of 12.8 was obtained in the absence of nematodes. Where both the fungus and the nematodes were added, an average wilt rating of 8.4 was obtained. This difference was found to be statistically significant.

Glassy end and glassy-end rot of potatoes in 1955. Smith, W. L., Jr., and P. H. Heinze. Glassy end (as well as glassy-end rot) appears to be associated with hot dry summers followed by wet falls. This disorder was found in Katahdin tubers from a field in Pennsylvania in 1955. It occurred mostly in abnormal potatoes characterized by field sprouts, new stolons, and various types of secondary

growths. The affected tissue in the stem end of the cut tubers was glassy, or watery, and its extent varied in different tubers. Frequently the main tuber, but not the secondary growth, was affected. Iodine tests showed that the glassy areas were devoid of starch. In storage tests at 40°, 55°, 75°, and 85°F, abnormal tubers sprouted more rapidly (within 2 weeks) than normal tubers, and a higher percentage of them had decay after 1 and 4 months. The decayed areas were watery. No undesirable odor was detectable in them unless secondary organisms had entered. A high percentage of abnormal tubers not developing decay at 40° or 50° had glassy end. At higher storage temperatures, a larger percentage of them developed decay. The combined percentages of tubers with glassy end and glassy-end rot, however, were essentially the same at all storage temperatures.

Stem cankers on oak associated with drought. TRUE, R. P., AND E. H. TRYON. Trunk cankers associated only with the drought year, 1953, were studied in 1955, chiefly on oaks of the red oak group on ridge-top sites near Morgantown, West Virginia. Other species less severely affected included Fire Cherry, Cucumber Magnolia, Red Maple, Black Cherry, and Chestnut Oak. There was a slight tendency for trees with suppressed crowns to be most severely affected. Cankers were most abundant on those red oaks and black oaks situated at or near the tops of ridges. No cankers were found on these species when growing on adjacent alluvial bottomland. Cankers were found on all sides, but were somewhat more abundant on the south sides of single-stemmed cankered trees of all species. Twin trees were more severely cankered than single-stemmed trees, and the sides of twin stems nearest each other were the most severely cankered. No recognized parasites were isolated from the sapwood of cankers. The cankers were associated with conditions of acute water shortage, but no explanation is here offered concerning how this sort of localized killing can result from drought.

Relation of temperature to transmission of the potato leafroll virus. WEBB, R. E. Aphids (*Myzus persicae* (Sulz.)) were reared at 22° and 27° on caged Katahdin potato plants infected with leafroll virus. Single adult apterous aphids were transferred with a camel's-hair brush to individual plants of *Physalis floridana* Rybd. and enclosed in a pliofilm cage for a 3-day feeding period. In 4 separate trials, 208 indicator plants were inoculated at 27°, and 217 were inoculated at 22°. At 27°, 58 per cent became infected; at 22°, 26 per cent became diseased. When individual aphids were transferred from virus-infected plants at 27° to 114 indicator plants at 22°, 50 per cent became infected. When aphids were transferred from leafroll-affected plants at 22° to 105 indicator plants at 27°, 32 per cent became infected. Twenty plants each of Katahdin, Chippewa, and X 927-3 were inoculated at 22° and at 27° by means of 15 viruliferous aphids per plant from infected plants at 22° and at 27°, respectively. At 22°, 11, 17, and 0 plants, respectively, became infected; at 27°, 16, 20, and 18 plants, respectively, became diseased. Apparently high temperature influenced the number of infective aphids and lowered resistance of the potato.

Control of several fungus diseases of beans and lima beans with antibiotics. ZAUMEYER, W. J., AND R. E. WESTER. Recent greenhouse tests showed that anisomycin, Mycostatin (fungicidin), oligomycin, griseofulvin, and Filipin protected beans and lima beans from 1 or more of the 4 diseases against which they were tested. Bean and lima bean seedlings were sprayed with aqueous solutions or suspensions of the antibiotics and 24 hours later inoculated with spore suspensions of the several fungi. The inoculated plants were placed in a fog chamber for 12–48 hours, depending upon the pathogen used, and later in an open greenhouse. Anisomycin protected beans from rust (caused by *Uromyces phaseoli* var. *typica*) and lima beans from downy mildew (caused by *Phytophthora phaseoli*). Mycostatin protected beans from anthracnose (caused by *Colletotrichum lindemuthianum*) and gave partial protection against bean rust. Oligomycin protected lima beans from downy mildew and stem anthracnose (caused by *C. truncatum*) and beans from rust and bean anthracnose. Griseofulvin protected beans from rust, whereas Filipin protected lima beans from downy mildew and partially protected against bean anthracnose; both were ineffective against infection by the other fungi. Rimocydin failed to protect beans and lima beans from infection by any of the pathogens tested. Anisomycin was the only antibiotic that eradicated bean rust, but it did not do so completely.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

DRACHOVSKA-SIMANOVA, MIROSLAVA. 1955. **Repne virosy a jejich prenaseci.** Statni Zemedelske Nakladatelstvi, Praha. 198 p. Price: paper, 30.65 Kcs.; cloth 37.20 Kcs. (A 32-p. mimeographed resume in German also is available.)

Detailed information on the virus diseases of beets is provided, with emphasis on practical information of value to the agronomist and the grower. Diseases are divided into 3 groups: yellows, mosaics, and curly top. For each disease, there are descriptions of symptoms and of the disease cycle; a discussion of the effect of environment on the disease; technical information on the causal agent and its transmission; and data on losses. Control methods are discussed at length, and specific recommendations are given. Major attention is given to sugar beets, both as grown for sugar and as grown for seed, but considerable information on diseases of table beets is presented.

DRACHOVSKA-SIMANOVA, MIROSLAVA. 1955. **Atlas repnych skudcu a chorob.** Vydava Ministerstvo Portravinarskeho Prumyslu, Praha. 47 color plates.

This atlas, devoted to the pests of beets, consists of 47 color plates on light cardboard, with brief information on the pests, the injuries they cause, and recommended control measures. The list includes insects, nematodes, fungi, viruses, and mineral deficiencies. The plates can be removed easily from the binder.